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THE PHILIPPINE
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SCREENING OF PHILIPPINE PLANTS FOR STEROIDAL SAPOGENINS, III

By FELICIDAD ESTORES ANZALDO, JOAQUIN MARAÑON, and SANTA F. ANCHETA
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Extreme interest has been drawn recently to the steroidal sapogenins as good precursors for the synthesis of sex hormones and cortisone. The brilliant researches of Marker and Applezweig(4) stimulated a survey of the plant kingdom, which has led to the screening of various kinds of plants. Collection was concentrated in particular geographical areas such as Mexico, Cuba, Chile, Peru, Turkey, Central and South America, South Africa, and the southeastern United States. In the Philippines, collection is being carried on in northern and southern Luzon.

This paper is a continuation of the previous reports(1) on different parts of plant samples representing fifty identified species collected for the investigation.

EXPERIMENTAL PROCEDURE

A rapid microprocedure reported by Monroe Wall, et al.(7) for the extraction and isolation of steroidal sapogenins and the identification methods of Henry Walens, et al.(6) were followed.

Extracts were prepared from different plant samples collected using 80 per cent ethyl alcohol as the solvent. The alcoholic extracts were tested for saponin content by hemolysis using standardized cow's blood suspended in normal saline solution.

Digitonin solution (10 mg of pure digitonin in 100 ml of 80 per cent ethyl alcohol) was used as the standard. The stock blood suspension was progressively diluted with 0.85 per cent sodium chloride solution, until 10 ml of the blood suspension was completely hemolyzed by 1 ml of digitonin solution at room temperature within 5 minutes. Samples were tested and those extracts giving a negative test were discarded.

A crude sapogenin sample was obtained by acid hydrolysis and alkali purification as described previously.(7) The total crude sample was dissolved in chloroform. An aliquot equivalent to approximately 0.1 gm was taken, evaporated to dryness in a tared beaker and the residue accurately weighed. From the value obtained the total dry weight of crude sapogenin was calculated. The residue was then acetylated, dried and weighed. The sapogenins were separated into monohydroxy and dihydroxy by adsorption chromatography using activated alumina as the adsorbing agent. An ultraviolet determination was then carried out and the percentage of steroidal sapogenins determined as previously calculated.(7)

RESULTS AND DISCUSSIONS

Table 1 gives the data for each sample. It includes the results of the hemolysis tests and the amount of steroidal sapogenins found in the plants under investigation.

TABLE 1.—*Results of hemolysis tests and estimated sapogenin content found in the plant collections.*

Species	Local name	Plant part	Hemolysis test	Estimated total m.f.b.
COMPOSITÆ				*Percent*
Achillea millefolium Linn.	Milfoil	Flowers	+	0
Artemisia vulgaris Linn.	Damong-maria	Leaves	+	0
Do.	do	Stems	+	0.19
Blumea balsamifera (Linn.) D. C.	Sambong	Leaves	+	0
Do.	do	Stems	+	0
Carthamus tinctorius Linn.	Kasubha	Flowers	+	0.08
Chrysanthemum indicum Linn.	Mansanilla	Whole plant	+	0.31
Chrysanthemum sinense Sabine	Rosas de japon	Leaves	—	0
Crossostephium chinense (Linn.) Merr.	Ajenjo	do	—	0
Do.	do	Stems	—	0
Eclipta alba (Linn.) Hassk.	Tinta-tinta	Leaves	—	0
Do.	do	Stems	—	0
Elephantopus mollis HBK.	Malatabako	Leaves	—	0
Elephantopus scaber Linn.	Dila-dila	do	—	0
Do.	do	Stems	—	0
Do.	do	Flowers	+	0.20
Helianthus annuus Linn.	Mirasol	do	—	0
Lactuca sativa Linn.	Letsugas	Leaves	+	0.18
Sphaeranthus africanus Linn.	Botobotonisan	do	—	0
Tagetes patula Linn.	Amarillo	do	—	0

TABLE 1.—*Results of hemolysis tests and estimated sapogenin content found in the plant collections*—Continued.

Species	Local name	Plant part	Hemolysis test	Estimated total m.f.b.
ANACARDIACEÆ				
Anacardium occidentale Linn.	Kasúi	Stems	—	0
Do.	do	Bark	+	0.319
Do.	do	Leaves	—	0
Mangifera indica Linn.	Mangga	do	+	0
Do.	do	Seeds	—	0
Do.	do	Stems	—	0
Do.	do	Bark	—	0
Do.	do	Flowers	+	0
Semecarpus cuneiformis Blanco	Ligas	Leaves	—	0
Do.	do	Stems	—	0
Spondias purpurea Linn.	Sineguelas	Leaves	—	0
Do.	do	Fruit	—	0
Do.	do	Bark	—	0
Do.	do	Stems	+	0
UMBELLIFERÆ				
Apium graveolens Linn.	Kintsay	Leaves	—	0
Do.	do	Stems	—	0
Centella asiatica (Linn.) Urban	Takip-kohol	Whole plant	+	0.23
Coriandrum sativum Linn.	Wansuy	Leaves	—	0
Do.	do	Stems	—	0
Daucus carota Linn.	Carrot	Tubers	+	0.52
Foeniculum vulgare Gaertn.	Anis	Fruit	+	0.12
CRUCIFERÆ				
Brassica integrifolia (West) O. E. Schulz	Mustasa	Leaves	+	0
Brassica oleracea Linn. var. *capitata* Linn.	Repolyo	Leaves	+	0.037
Brassica chinensis Linn.	Petsay	Stalk	+	0
Do.	do	Leaves	+	0
Raphanus sativus Linn.	Labanos	Stalk	+	0
Do.	do	Tubers	+	0.74
LAURACEÆ				
Cassytha filiformis Linn	Kawad-kawaran	Leaves	+	0
Do.	do	Stems	+	0
Cinnamomum mercadoi Vidal	Kalingag	do	+	0.086
Do.	do	Leaves	+	0.63
Cinnamomum zeylanicum Blume	Canela	Bark	—	0
Litsea glutinosa (Lour.) C. B. Rob.	Puso-puso	Leaves	—	0
Do.	do	Stems	—	0
Do.	do	Bark	—	0
Persea americana Mill.	Abokado	Seeds	+	0
Do.	do	Leaves	—	0
Do.	do	Stems	—	0
Do.	do	Bark	+	0.30
MUSACEÆ				
Musa errans (Blanco) Teodoro var. *botoan*	Saguing (Butuan).	Fruit	—	0
Do.	do	Leaves	—	0
Do.	do	Stalk	—	0
Do.	do	Peel	—	0
Musa paradisiaca var. *maxima*	Saguing (Batavia).	Fruit	+	0
Do.	do	Peel	—	0
Musa sapientum Linn.	Saguing (Saba)	Fruit	+	0.34
Do.	do	Leaves	—	0
Do.	do	Stalk	—	0
Do.	do	Peel	—	0
Musa sapientum var. *cinirea*	Saguing (Latundan).	Leaves	—	0
Do.	do	Fruit	—	0
Do.	do	Peel	—	0
Do.	do	Stalks	—	0
Musa sapientum var. *lacatan*	Saguing (Lacatan).	Leaves	—	0

TABLE 1.—*Results of hemolysis tests and estimated sapogenin content found in the plant collections*—Continued.

Species	Local name	Plant part	Hemolysis test	Estimated total m.f.b.
Musa sapientum var. *lacatan*	Saguing (lacatan)	Fruit	—	0
Do.	------do------	Peel	—	0
Do.	------do------	Stalk	—	0
Musa sapientum var. *suaveolens*	Saguing (Buñgulan).	Leaves	—	0
Do.	------do------	Peel	—	0
Do.	------do------	Fruit	—	0
Do.	------do------	Stalk	—	0
OXALIDACEÆ				
Averrhoa bilimbi Linn.	Kamyas	Leaves	—	0
Do.	------do------	Stems	—	0
Do.	------do------	Bark	—	0
Do.	------do------	Fruit	+	0.16
Averrhoa carambola Linn.	Balimbing	Leaves	—	0
Do.	------do------	Bark	+	0.19
Do.	------do------	Stems	—	0
Do.	------do------	Fruit	—	0
Biophytum sensitivum (Linn.) D. C.	Damong-bing-kalat.	Leaves	—	0
Oxalis repens Thunb.	Taiñgan-daga	Leaves and stems	—	0
CONVOLVULACEÆ				
Argyreia nervosa (Burm. f.) Boj.	Sedang-dahon	Leaves	—	0
Ipomea aquatica Forsk.	Kangkong	------do------	—	0
Do.	------do------	Stems	—	0
Ipomea batatas (Linn.) Poir.	Kamote	Leaves	—	0
Do.	------do------	Stems	—	0
Do.	------do------	Tubers	—	0
Do.	------do------	Roots	+	0
Ipomea hederacea (Linn.) Jacq.	Kamokamotihan	Leaves	—	0
Merremia umbellata (Linn.) Hallier f.	Kalamitmit	------do------	+	0
Quamoclit pennata (Desr.) Bojer	Cabello de angel	------do------	—	0

A positive hemolysis test for saponin was obtained in about 60 per cent of the species. Steroidal sapogenins were found in 5 species of the Compositæ family, 3 species of the Lauraceæ, 3 of the Umbelliferæ, 2 of the Cruciferæ and Oxalidaceæ and 1 of the Anacardiaceæ and Musaceæ families. Plant samples under the family Convolvulaceæ were found to be negative.

A few new records of high content of sapogenins are reported in labanos (*Raphanus sativus* Linn.) tubers, kaliñgag (*Cinnamomum mercadoi* Vidal) leaves, and carrot (*Daucus carota* Linn.) tubers.

SUMMARY

Plants under the family Compositæ, Anacardiaceæ, Umbelliferæ, Cruciferæ, Lauraceæ, Musaceæ, Oxalidaceæ, and Convolvulaceæ were investigated. Alcoholic extracts were made from the plant samples collected. These extracts were examined for

the presence of saponin using the hemolysis test. Almost 60 per cent of the extracts gave positive results but only seventeen samples gave spectral curves of the steroidal sapogenins. Table 1 shows that the tubers of *Raphanus sativus* Linn. and *Daucus carota* Linn., and the leaves of *Cinnamomum mercadoi* Vidal gave promising results.

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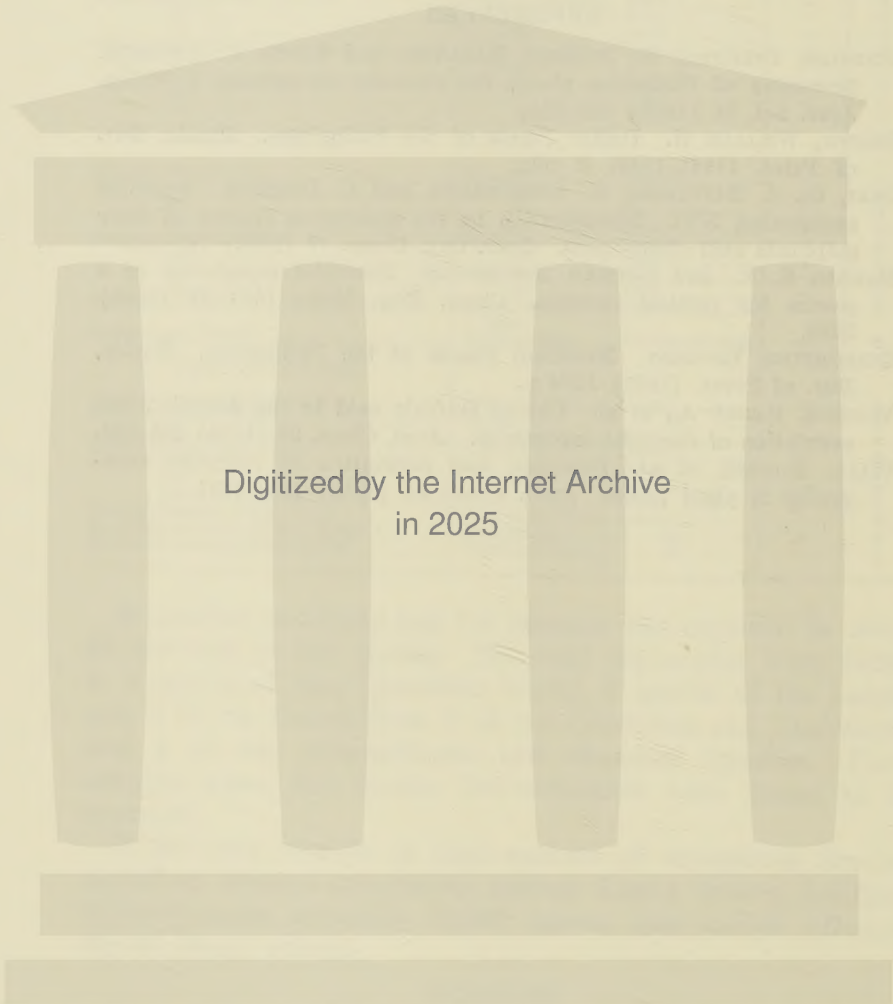

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STUDIES ON PARAGONIMIASIS, IV *

A SURVEY IN CASIGURAN, SORSOGON, PHILIPPINES

By Mariano G. Yogore, Jr., Benjamin D. Cabrera, and Glenn A. Noble [1]
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ONE TEXT FIGURE

A survey to obtain epidemiologic data on paragonimiasis in the Philippines was conducted by the authors in three barrios of the Municipality of Casiguran, Sorsogon Province, Luzon Island in April, 1954. Casiguran was chosen for the study because Tubangui in 1948–1949 had found a high percentage of crabs collected from a stream in one locality (Barrio Mabini) within this municipality positive for *Paragonimus* metacercariæ.

With full cooperation and assistance from provincial and municipal administrative and health officials, we set up our headquarters in the Sorsogon Provincial Hospital at the provincial capital of Sorsogon. One important factor, however, hampered the investigation—the presence of communist dissidents in the survey area at the time. This circumstance limited both our movement within the area and contact with the barrio folk to daylight hours and, for a while, a military escort was ordered for us by the Philippine Constabulary.

The work in this survey involved:

(1) Collection and examination of crabs and snails for the larval stages of *Paragonimus;*
(2) Collection of feces and sputum from the people and examination of these for *Paragonimus* ova; and
(3) Autopsy examinations of dogs and cats for infection with *Paragonimus.*

THE STUDY AREA

The Municipality of Casiguran is found on the western shore of Sorsogon Bay. The terrain is flat only close to the shoreline, and becomes rapidly hilly and mountainous away from the coast toward the mountain ranges situated at the center of the province.

* Studies supported by generous research grants from the University of the Philippines. Dr. Noble's expenses for this survey were paid for by the American Academy of Arts and Sciences.

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Typical of the organization of municipalities in the Philippines, Casiguran consists of a center of population called the "poblacion," where the local government building, the school, the church, etc, are found; and outlying "barrios," which are minor centers of population. Casiguran has a population of about 13,000, of which about 4,000 live in the poblacion and 9,000 in 15 barrios. No detailed map of the municipality was available but a sketch map, including population estimates, provided us by the provincial health officer of Sorsogon is reproduced in Fig. 1. Casiguran is almost exclusively agricul-

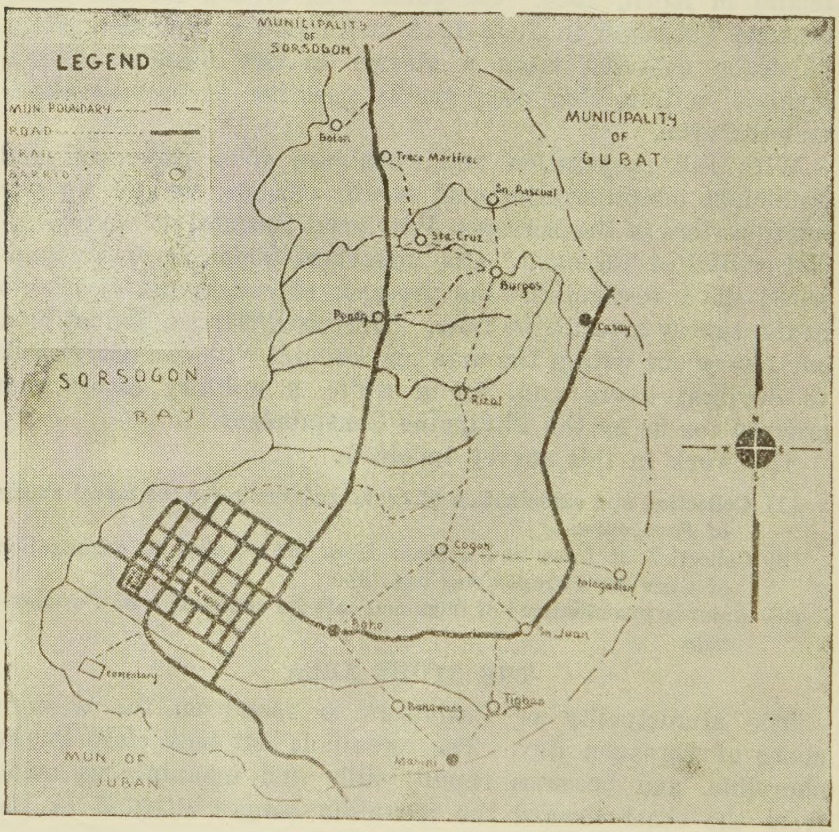

FIG. 1.

tural. Coconut, abaca, and rice are raised in this municipality and the income of most of the inhabitants depend on the planting and raising of these and on the processing of coconut into copra and of the stripping of abaca into abaca fiber for Manila hemp.

The rainfall in Casiguran and its vicinity averages about 140 inches a year, with the heaviest fall from October to January, when more than 20 inches may fall in one month. There is no dry season, and the months from March to May, which have the least rainfall, average 5 or 6 inches of rain each. There are about 160 rainy days each year.

The three barrios of the municipality chosen for this study were Barrio Mabini (estimated population, 380), Barrio Boho (estimated population, 350), and Barrio Casay (estimated population, 250). Boho is located within 3 km of the poblacion, and, being closer to the coast, has a less rolling terrain than Mabini or Casay. Casay is in rugged, mountainous country and is about 8 km distant from the poblacion. Mabini is about 10 km from the poblacion and has a terrain intermediate between that of Boho and Casay. Located about 2 km from the provincial highway, Mabini can be reached only by a rough trail which however can be travelled by jeep.

A collection of houses along the provincial road form the center of Barrio Boho. Other houses are located far from the road. On each side of the barrio center, running parallel to the road are two streams—Gimaoyon and Buracbusac. The same set-up is found in Casay, with the streams named Casay and Cabatagon. The center of Barrio Mabini is a group of houses arranged around the quadrangle of a two-room schoolhouse and its playground. Two streams, named Rangas and Aruroy, run through the barrio.

The living conditions of the people in these barrios are comparable. The people are poor, barely eking out a living from the soil, and they live in bamboo and "nipa" (a palm tree) houses, without sanitary facilities of any kind. There are no toilets, the people defecate in the fields. Water for drinking and other domestic purposes must be fetched from the streams. Coconut trees dominate the land, and big plantations of abaca are common.

MATERIALS AND METHODS

STUDIES ON THE INTERMEDIATE HOSTS OF PARAGONIMUS

A survey of each stream in the three barrios was made to note its physical characteristics and to determine whether or not the snail, *Brotia asperata,* was present. Since previous preliminary work by Tubangui, et al.(6) indicated that a very low percentage of these snails could be expected to be positive

for *Paragonimus* larval stages, we were prepared to examine hundreds of the snails to obtain a good index of infection. It was decided to limit collection and examination of the snail host from only two streams where a high percentage of the crabs would be found positive for *Paragonimus* metacercariæ on preliminary examination. Accordingly, *Brotia asperata* only from Aruroy Stream in Mabini and Gimaoyon Stream in Boho were examined.

Examination of the snail.—The technique used in examining *Brotia asperata* for larval stages of *Paragonimus* has already been described.(11)

Examination of the crab.—Two procedures were employed for the examination of *P.* (*B.*) *grapsoides* for *Paragonimus* metacercariæ.

1. Rapid examination. The carapace was separated from the rest of the body, then the heart and pericardium and the gills were inspected grossly for the metacercariæ.
2. Detailed examination. The heart and pericardium, gills, liver, and body muscles were dissected out and prepared as press preparations between slides. The examination for and counting of metacercariæ were done under a dissecting microscope at 10x.

STUDIES ON THE HUMAN POPULATION

Contact with the people of the three barrios was established through group meetings and by house to house visits. During such contact, specimen containers for sputum and feces were distributed to all persons seven years of age or older, instructions on the method of collection were given, and information of such pertinent matters as eating habits was obtained. A responsible individual was asked to collect and deliver the specimens to the survey team on the day after the distribution of the containers.

Sputum examination.—Sputum specimens were examined both by direct smear and by concentration. In the concentration procedure, 3 per cent sodium hydroxide was added to the specimen, the mixture was allowed to stand for 30 minutes with occasional shaking, and then centrifuged. The supernate was decanted and a few drops of the sediment was examined microscopically.

Feces examination.—Fecal specimens were examined by the 40 per cent hydrochloric acid-ether centrifugation technic as described by Weller and Dammin.(7)

AUTOPSIES OF CATS AND DOGS

A few cats and dogs obtained from two of the barrios were killed and the lungs examined for the presence of adult *Paragonimus*. An examination for *Paragonimus* ova by the acid-ether technic of feces obtained from the large intestine of the autopsied animal was also performed.

RESULTS AND OBSERVATIONS

THE STREAMS IN THE STUDY AREA

In all of the six streams of the three barrios, *Brotia asperata* and *Parathelphusa (Barythelphusa) grapsoides* were present. *P. (B.) grapsoides* infected with *Paragonimus* metacercariæ were likewise found in all streams.

Aruroy Stream—4 to 6 feet wide and about 2 feet deep in most parts. Rocky stream bed with big boulders. Very steep banks. Densely shaded in some parts. Cool, very clear, swiftly flowing water.

Rangas Stream—10 to 15 feet wide and 2 to 3 feet deep. Sandy stream bed with small stones. Gently sloping banks. Partly shaded. Cool, very clear, swiftly flowing water.

Gimaoyon Stream—4 feet wide and one foot deep. Sandy bed with small stones and few boulders. Very steep banks. Shaded. Cool, clear, swiftly flowing water.

Buracbusac Stream—15 to 20 feet wide, up to 5 feet deep. Sandy and muddy bed with small stones. Flat banks. Unshaded. Cool, swiftly flowing, slightly muddy water.

Casay Stream—About 6 feet wide, a few feet deep. Sandy bed with big boulders. Steep banks. Partly shaded. Clear, swiftly flowing water.

Cabatagon Stream—About 10 feet wide, one foot deep. Similar in all respects to Casay Stream.

THE INTERMEDIATE HOSTS

The molluscan host.—No attempts were made to measure the density of *Brotia asperata* in the streams of the study area. It was observed, however, that these snails were abundant in Aruroy and Gimaoyon streams. In some portions of these streams, one could stay put in one spot and have within arm's reach as many as 20 to 30 snails.

Of 1,000 *Brotia asperata* from Aruroy stream of Mabini, 7 or 0.7 per cent were found positive for cercariæ and second generation radiæ of *Paragonimus*. Of 350 *Brotia asperata* from Gimaoyon stream of Boho, 2 of 0.57 per cent were found positive.

The crustacean host.—A total of 137 crabs were collected from the six streams of the study area and examined for *Paragonimus* metacercariæ. As far as we could tell, all these crabs belonged to one species, later identified as *Parathelpusa (Barythelphusa) grapsoides.*(10) The results of the examination of these crabs are given in Table 1.

TABLE 1.—*Infection of P. (B.) grapsoides from Casiguran, Sorsogon, with Paragonimus metacercariæ.*

Stream	Date of collection (1954)	Number examined	Number positive	Per cent infected	Number of metacercariæ in positive crabs (Total from 4 tissues *)
MABINI:					
Aruroy	April 2	8	6	75.0	-1-1-2-3-81-87
	April 5	24	3	12.5	-4-63-77
Rangas	April 5	22	6	27.3	-1-3-6-6-18-20
TOTAL		54	15	27.8	
BOHO:					
Gimaoyon	April 9	6	2	33.3	-1-2
	April 10	6	6	100.0	-2-125-152-281-440-636
	April 16	10	7	70.0	-1-3-5-9-25-37-37
	April 21	24	24	100.0	not determined
Buracbusac	April 9	3	0	0.0	
	April 10	17	12	70.6	-1-6-8-13-14-16-19-23 -389-800 ?-**-**
	TOTAL	66	51	77.3	
CASAY:					
Casay	April 13	10	3	30.0	-1-1-26
Cabatagon	April 13	7	4	57.1	-1-1-1-12
TOTAL		17	7	41.2	

* Heart and pericardium, gills liver and body muscles.

? Very numerous metacercariæ in this crab; figure given is an estimate.

** Metacercariæ not counted.

The crabs from Boho showed a significantly higher average percentage of infection than the crabs from Casay and Mabini, and had a heavier metacercarial load as well. There was no significant difference between the percentages of infection in Mabini and Casay, but the crabs from Mabini appeared to have a heavier load.

The results of counting the *Paragonimus* metacercariæ in the heart and pericardium, gills, liver and body muscles of 41 positive *P. (B.) grapsoides* are given in Table 2.

TABLE 2.—*Distribution and number of Paragonimus metacercariæ in P. (B.) grapsoides from Casiguran, Sorsogon.*

Crab number	Number of *Paragonimus* metacercariæ				
	Total in four tissues	In heart and pericardium	In liver	In gills	In body muscles
1	1	1	0	0	0
2	1	1	0	0	0
3	1	1	0	0	0
4	1	1	0	0	0
5	1	1	0	0	0
6	1	0	1	0	0
7	1	0	0	1	0
8	1	0	0	1	0
9	1	0	0	0	1
10	1	0	0	0	1
11	1	0	0	0	1
12	2	1	0	1	0
13	2	1	0	0	1
14	2	0	0	1	1
15	3	2	0	0	1
16	3	1	0	1	1
17	3	0	0	0	3
18	4	0	0	4	0
19	5	3	0	1	1
20	6	2	0	0	4
21	6	1	0	4	1
22	8	7	0	1	0
23	9	9	0	0	0
24	12	7	0	3	2
25	13	7	1	0	5
26	14	11	1	0	2
27	16	9	0	2	5
28	18	3	0	6	9
29	19	16	0	0	3
30	20	10	0	0	10
31	23	20	1	0	2
32	25	23	1	0	1
33	26	8	3	0	15
34	37	31	2	2	2
35	37	36	0	1	0
36	125	40	4	25	56
37	152	71	4	18	59
38	281	90	125	22	44
39	389	230	32	43	84
40	440	108	279	18	35
41	636	491	12	45	88
Total	2,347	1,243	466	200	438
Per cent of total by tissue		(52.96)	(19.86)	(8.52)	(18.66)
Number of crabs positive by tissue		31/41	13/41	20/41	28/41
Per cent of crabs positive by tissue		(75.6)	(31.7)	(48.8)	(68.3)

Paragonimus metacercariæ were found in the heart and pericardium of 31 or 75.6 per cent of these crabs; in the body muscles in 28 or 68.3 per cent; in the gills in 20 or 48.8 per cent and in the liver in 13 or 31.7 per cent. Of a total of 2,347 *Paragonimus* metacercariæ counted in these four tissues of the 41 crabs, 1,243 or 52.96 per cent were found in the heart and pericardium; 466 or 19.86 per cent were in the liver; 438 or 18.66 per cent were in the body muscles and 200 or 8.52 per cent were in the gills.

THE HUMAN POPULATION

Eating habits in relation to paragonimiasis.—The inhabitants of the three barrios readily admitted eating crabs caught from the streams. These crabs are an important supplement and a common item in their diet. They, however, strongly denied eating these crabs in the raw or insufficiently cooked condition.

The most common method of preparing the crabs for the table is by thorough boiling in water. The juice of the crab is also used in the preparation of a dish called "kinagang." "Kinagang" is prepared as follows:

> The raw crabs are first chopped into small pieces and then ground by mortar and pestle. The ground crab tissue is placed inside a piece of cloth and then squeezed and the crab juice collected. The juice is added to the meat of grated coconut and the mixture is wrapped in some leaves. The leaf-wrapped mixture is then boiled until dry.

Sputum and feces examination.—The response of the people to the request for sputum and fecal specimens may be gauged from Table 3. Two hundred and seventy-five persons, representing 28 per cent of the estimated population of the three barrios, submitted specimens for examination.

TABLE 3.—*Collection of sputum and fecal specimens from three barrios of the study area.*

Barrio	Estimated population	Total number of specimens submitted		Feces and sputum specimens submitted		Sputum specimens only submitted		Fecal specimens only submitted	
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Mabini	380	138	36.3	37	9.7	57	15.0	44	11.6
Boho	350	99	28.3	16	4.6	40	11.4	43	12.3
Casay	250	38	15.2	23	9.2	0	0.0	15	6.0
Total	980	275	28.1	76	7.8	97	9.9	102	10.4

Only 2 individuals or 0.7 per cent were found infected with *Paragonimus*. One was a 10-year-old female from Barrio Mabini, the only one of 138 persons from this barrio who was found positive. The other infected individual was a 60-year-old male from Barrio Boho, who was the only one of 99 persons from this barrio who was found positive. *Paragonimus* ova were found in both the feces and sputum of the child, while only the sputum of the old man was positive on the first examination.

On repeat examinations, *Paragonimus* ova were also demonstrated in the feces of the old man. The sputum specimens submitted by the positive child had blood streaks; that of old man had no gross evidence of blood but a history of several attacks of hemoptysis was elicited from this individual. The sputum specimens from both were negative for acid-fast organisms.

Findings incidental to the survey for paragonimiasis but revealing of the living conditions in these barrios may be mentioned. Of 177 persons from the three barrios who submitted fecal specimens, 95.4 per cent were infected with *Trichuris*, 89.8 per cent had hookworms, and 71.8 per cent were positive for *Ascaris*.

OTHER DEFINITIVE HOSTS OF PARAGONIMUS

It was only after the disappointingly (to us) low number of positives had been found on examination of sputum and feces from the human population that a search for other definitive hosts of *Paragonimus* was undertaken.

Two cats and two dogs from Barrio Mabini and four cats from Barrio Boho were killed and their lungs examined. Both dogs were negative for *Paragonimus* but the two cats from Mabini were positive. There were nine cystic lesions of paragonimiasis in the lungs of one cat and five in the other. Two of the four cats from Barrio Boho were found infected with *Paragonimus*—there were nine cystic lesions in the lungs of one cat and ten in the other. Numerous *Paragonimus* ova were seen in the feces taken from the large intestines of all four positive cats.

DISCUSSION

Until other intermediate hosts are discovered, paragonimiasis in the Philippines may be regarded as limited to those areas of the country where *Brotia asperata* and *Parathelphusa (Barythelphusa) grapsoides* are found. The physical characteristics of streams that offer a natural and favorable habitat for these intermediate hosts have already been described.(10) Such streams are to be found in the foothills where the terrain is rough and rolling, and more in inland localities but also near the coast in those areas where the base of mountains may extend to near the sea. There is no dearth of localities in this country which will provide a favorable habitat for these intermediate hosts. Until information to the contrary becomes

available, if ever, *Paragonimus* in the Philippines may therefore be thought of as the "hilly breeder" type, in contradistinction to the "plain breeder" type, in the classification suggested by Chen.(1)

Coupled with the presence of suitable intermediate hosts for *Paragonimus*, the setting for paragonimiasis in the three barrios of Casiguran include an interplay of favorable climatic conditions and a substandard way of life of the people which has resulted in a very high prevalence of the common nematode infections, as revealed by the fecal studies. Among these impoverished and generally uneducated people who live without the most basic sanitary facilities, the streams where the intermediate hosts of *Paragonimus* abound assume a vital importance not only as a source of water for drinking and other purposes but also as a source of food in the form of snails, crabs, and shrimps.

INFECTION IN BROTIA ASPERATA

The very low infection rate in the snail intermediate host (0.7 per cent in Aruroy Stream, Barrio Mabini and 0.57 per cent in Gimaoyan Stream, Barrio Boho) observed in this study is in consonance with the observation of Tubangui, et al.(6) who reported only two infected individuals out of 1,986 from Aruroy Stream. Another chance to check snail infection in Aruroy Stream presented itself during our study of the detailed larval morphology. In a December, 1955 collection of *B. asperata*, only 4 (0.13 per cent) of 3,000 were found positive for *Paragonimus* larvæ.

The observation of a very low rate of *Paragonimus* infection in the snail host with a very high rate in the crustacean host is in agreement with data from two endemic areas for paragonimiasis in Japan and represents apparently the usual situation. In Shizuoka Prefecture, where as high as 94 per cent of crabs (*Eriocher japonicus*) were found to be infected(3, 9) the infection rate in the important snail host (*Semisulcospira libertina*) was only 2.8 per cent(8) and this is the highest figure reported in Japan. In Ehime Prefecture, where the crab infections were of the same order as in Shizuoka,(5, 7) infection in the snail *Semisulcospira japonica* (a variety of *S. libertina*) averaged only 0.2 per cent.(8)

It must be surmised, therefore, that a low infection rate in snails is sufficient to maintain a high rate of infection in

crabs. Obviously, the relative number of snails and crabs in the same stream, as well as the effectiveness of a single infected snail as a source of infection of crabs, must be considered in the snail-crab relationship. There was no way of judging how many crabs may be infected from one snail source in the two Casiguran streams studied, but it was evident that even with the low percentage of infection in the snails, quite a number of infected snails must have been present in these streams since the snails were so numerous.

INFECTION IN PARATHELPHUSA (BARYTHELPHUSA) GRAPSOIDES

Prevalence and density of infection.—In all six streams studied in Casiguran, *P.* (*B.*) *grapsoides* harboring *Paragonimus metacercariæ* were found. Measurement of the prevalence and density of infection in the second intermediate host is of great importance in the epidemiology of paragonimiasis because it indicates the infective potential to the definitive host.

Successive collections of the crab from the same stream showed us that there was a rather wide variation in infection rate as well as in metacercarial load. This finding suggests that opportunities for contact between the crab and the cercariæ of *Paragonimus* may vary widely at different portions of the same body of water at the same and different times and it may well be that opportunity for such contact may occur only in small foci in the stream. The interplay of a great number of factors governing the movement of the infected snail, the cercaria and the crab will obviously affect both the prevalence of infection in the crab and the metacercarial load.

In spite of the inconsistency in crab infection rates and metacercarial load that we obtained, it is noteworthy that some lots of crabs showed 100 per cent infection and that some crabs harbored metacercariæ in the hundreds. These observations in *P.* (*B.*) *grapsoides* may be compared with that in *Eriocheir japonicus*, the most important crab host for *Paragonimus* in Japan. An average of 72 per cent of *E. japonicus* from the endemic area of Shizuoka Prefecture was found infected by Yokogawa,(9) but there was a seasonal fluctuation with the infection rate being above 90 per cent in September and October and as low as 10 per cent in July. An infection rate of 95 per cent was observed by Takahashi(5) in an October-November collection of *E. japonicus* from Ehime Prefecture. The metacercarial load in *E. japonicus*, which was determined

by an examination of more tissues of this crab than was done for *P.* (*B.*) *grapsoides*, ranged from one to several hundred. From the standpoint of the infective potential to the definitive host, therefore, the infection in the crabs from Casiguran may be considered roughly similar to that in the crabs from Shizuoka and Ehime in Japan in the sense that a definitive host eating these crabs in the raw or inadequately cooked condition runs a very great risk of infection with *Paragonimus*.

Localization of Paragonimus metacercariæ.—Because the relatively tough body muscles of *P.* (*B.*) *grapsoides* did not lend itself well to examination for metacercariæ as press preparation (the technic we used in this study), we feel that we did not have accurate results in the counting of metacercariæ in this portion of the crab body. The figures in Table 2, however, emphasize one marked difference between the localization of metacercariæ in *P.* (*B.*) *grapsoides* as compared with that in *Eriocheir japonicus*. In our study, a high percentage (75.6 per cent) of 41 positive crabs had metacercariæ localized in the heart and pericardium, while only 48.8 per cent of these crabs had metacercariæ localized in the gills. Also, the number of metacercariæ found in the heart and pericardium of these positive crabs outnumbered those found in the gills by a ratio of about 6:1. Observations by Japanese workers on *E. japonicus* show an exactly opposite localization. In *E. japonicus*, metacercariæ are found in the gills of almost all positive crabs while metacercariæ almost never localize in the heart and pericardium. Thus, of 75 positive *E. japonicus* from Shizuoka Prefecture examined by Yokogawa,(9) 75 (100 per cent) were found to have metacercariæ in the gills, while none, (0 per cent) had metacercariæ in the heart and pericardium. Of 41 positive *E. japonicus* from Ehime Prefecture examined by Takahashi,(5) 41 (100 per cent) had metacercariæ in the gills while only a single crab (2.4 per cent) was positive in the heart and pericardium, and this consisted of only one metacercaria.

This finding on the difference in localization of *Paragonimus* metacercariæ between that of the crab host in the Philippines and in Japan not only brings out interesting questions on the host (crab)-parasite relationship but also has an important bearing on the conduct of crab surveys for infection with *Paragonimus* which may be undertaken in other areas of the Philippines in the future.

INFECTION IN THE HUMAN POPULATION

After our studies on the crab host, we felt quite sure that we would obtain a high paragonimiasis prevalence rate among the people of these barrios. We held this belief in spite of the strong assertion by these people that they did not eat these crabs in the raw or inadequately cooked condition. Examination of the sputum and/or fecal specimens from 275 persons of these barrios, however, showed only two positive individuals, an infection rate of only 0.7 per cent for the group. With this finding, and in the light of the intense infection we had observed in the crabs, we became faced with two possibilities: (*1*) That paragonimiasis was really highly prevalent among these people but that our examinations were so inefficient as to result in a great number of "missed" cases, or (*2*) That we give credence to the assertion that these people did not eat raw or inadequately cooked crabs and therefore accept that, as indicated by the results of the sputum and fecal examinations, paragonimiasis was at a minimal level in the human population. The second possibility carries with it the implication that some definitive host(s), other than man and with a high infection rate, must exist in these barrios.

Even under excellent conditions of examination, sputum and fecal examinations for *Paragonimus* ova will not reveal all cases of paragonimiasis. The limitations inherent in such examinations were revealed in a study by Komiya, et al.(2) In a study of 35 patients with paragonimiasis, in which a concentration examination of 24 hours sputum and a very efficient fecal concentration technic was used, these workers found that (*a*) only 37 per cent of the patients always showed ova in the sputum while the majority were sometimes positive and sometimes negative; (*b*) when the sputum was typical (viscuous, rusty-brown), *Paragonimus* ova were demonstrable in about 93 per cent of such specimens, while when the sputum was atypical this figure dropped to 24 per cent; and (*c*) only about one-fourth of the patients showed ova in the feces while the rest may or may not be positive on stool examination at different times.

We must admit that conditions for examination in the field were far from excellent and that while we used concentration technics for both sputum and stool specimens, our sputum samples probably represented only a few hours of collection

instead of 24. In addition, there was no repeat examination for the negative individuals. We are forced to conclude from these circumstances that our examinations must have brought to light only a small fraction of the actual number of infections among the people examined. Using the lowest efficiency figure obtained by Komiya, et al. (i.e., that only 24 per cent of atypical sputum specimens from paragonimiasis cases are positive for *Paragonimus* ova), we may multiply 0.7 per cent by 5 and obtain 3.5 per cent as probably indicating more accurately the prevalence of paragonimiasis in these barrios. It would seem safe for us, therefore, to assume that the paragonimiasis prevalence rate in this locality is less than 5 per cent.

With such a low paragonimiasis prevalence among these people, it becomes easy to accept the statement that these people, in general, do not eat raw or inadequately cooked crabs. This is logical, since if the eating of raw or inadequately cooked crabs were a widespread practice among these people, the high rate and density of *Paragonimus* infection in the crabs should lead to a much higher prevalence rate.

How may we account for the occurrence of paragonimiasis in some individuals of these barrios, few as they might be? An obvious answer to this question can be made on the assumption that a certain number of the population do not conform with the eating habits of the majority and that these persons do occassionally eat raw or half-cooked crabs with viable metacercariæ.

While the above explanation may suffice, a less obvious mechanism of infection in a few individuals may also be invoked with the benefit of hindsight gained from information which did not come into our possession until long after we had finished our survey. A similar situation to that we found in Casiguran faced Komiya, et al.(3) in their investigation of the endemic area of Shizuoka Prefecture. In that area, the crabs (*E. japonicus*) show a high rate of infection and a heavy metacercarial load, paragonimiasis is found in the human population at a varying rate of 2 per cent to 16 per cent but all the methods of preparing the crab for the table were found to involve procedures that could not leave viable metacercariæ. The observation that

E. japonicus was always eaten in the cooked condition was so firmly established that another Japanese investigator even questioned the significance of eating these crabs as a method of transmission of the parasite to the human population, while Komiya, et al. remarked:

> . . . the infection frequency and concentration of metacercariæ in *E. japonicus* of this district are very high, so that, if commonly eaten raw or inadequately cooked the infection rate of the inhabitants should have been much higher than it is now, and the severity of the infection much greater.

On detailed inquiry as to the eating habits of the people in this endemic area, Komiya and his co-workers found that the crab was most commonly eaten as "crab soup," which requires the boiling of the crab juice and tissues for about 20 to 30 minutes. In the preparation of "crab soup," however, the raw crabs are chopped up and crushed first on a block with a knife, and then in a mixing bowl. In observing the actual preparation of the soup, these workers were able to demonstrate *Paragonimus* metacercariæ on the hands of the cook, on the knife and chopping block that were used, and in other locations. These findings led these workers to think that infection of man in the Shizuoka endemic area is a result of contamination of food usually eaten raw (such as vegetable pickles and spices) with *Paragonimus* metacercariæ from the hands of the cook or from the same chopping block and knife and other kitchen utensils which had been previously used in the preparation of "crab soup."

A striking similarity is found in the preparation of "kinagang" and "crab soup" in the initial stages. It may therefore well be that at least a portion of the infection of man in Casiguran is accidental and results from the operation of a mechanism similar to that in Shizuoka.

INFECTION IN DOMESTIC CATS

Because we had found a very low prevalence of paragonimiasis in the human population in spite of the intense infection in *P.* (*B.*) *grapsoides,* we were forced to consider the possibility that other mammals might be serving as definitive hosts of *Paragonimus* in the study area. The possibility of a reservoir host in the epidemiology of paragonimiasis in this country has been but little considered heretofore. Only one report in the literature, that of Musgrave,(4) mentions the

occurrence of *Paragonimus* in a definitive host other than man in this country. Musgrave, fifty years ago, examined 32 cats, 23 dogs and 10 rats and mice (all presumably obtained in Manila) and found one infected cat.

The ease with which we found infected cats from barrios Boho and Mabini, in spite of the very limited number we examined, strongly indicated that a high percentage of these animals in the study area are infected with *Paragonimus*. While we cannot at this time completely assess the place of the domestic cat in the ecology of paragonimiasis in this locality, this observation certainly suggests that here the domestic cat is a more usual host of *Paragonimus* than man.

The domestic cat is not notably aquatic and whether it acquires its infection with *Paragonimus* by catching crabs in the streams or by waiting in the houses for portions of the crab that are thrown away by the people has still to be studied. It is possible that the cat may be a blind alley in the life cycle of *Paragonimus* in these barrios since its defecation habits do not usually bring it near the water. On the other hand, it is known that *Paragonimus* may maintain itself exclusively as an enzootic, as *P. kellicotti* is maintained in some parts of the United States in a mink (or other animal)-snail-crayfish-mink cycle. Thus, in localities in the Philippines where the crabs are not eaten in the raw or inadequately cooked state by the human population, as in Casiguran, a cat-snail-crab-cat cycle may be primary, with man coming into the biological cycle of the parasite only by accident.

SUMMARY AND CONCLUSIONS

(1) A survey to gather epidemiologic data on paragonimiasis in the Philippines was conducted in three barrios of the Municipality of Casiguran, Sorsogon Province, in April, 1954. The endemicity of paragonimiasis in the study area was established. The barrios are located in hilly terrain and have a climate characterized by very heavy rainfall and no dry season. The people involved are poverty-striken, uneducated farmers and their families who are dependent on the growing and processing of coconut and abaca for their livelihood. They live under the most primitive rural conditions without the basic sanitary facilities; consequently, the common intestinal roundworms infect almost all these people. The streams in their barrios, in which the intermediate hosts of *Paragonimus* are found,

play a most important role in their lives, providing them with water for drinking and other purposes and with supplementary food.

(2) Each barrio studied had two streams which provided a favorable habitat for the implicated intermediate host species. The snail, *Brotia asperata* and infected crabs, *Parathelphusa* (*Barythelphusa*) *grapsoides*, were found in the streams.

(3) A very low percentage (less than 1 per cent of *B. asperata* collected from two streams was found infected with *Paragonimus*. The low infection rate in these snails is comparable to the infection rate in *Semisulcospira libertina*, the important snail host of *P. westermani* in Japan.

(4) In spite of the low infection rate in the snail host, the crabs were observed to have a variable but generally high infection rate (up to 100 per cent in certain lots of crabs). The number of metacercariæ in infected crabs showed great variability; most of the infected crabs had less than 50 metacercariæ while some had more than 100. Infection in these crabs is roughly comparable to that in *E. japonicus*, the important crab host of *P. westermani* in Japan, in the sense that a definitive host in the habit of eating these crabs in the raw or inadequately cooked condition will most likely become infected.

(5) Localization of *Paragonimus* metacercariæ in *P.* (*B.*) *grapsoides* was observed to be remarkably different from that in *E. japonicus* in some respects. 75.6 per cent of 41 positive *P.* (*B.*) *grapsoides* showed metacercariæ in the heart and pericardium while only 48.8 per cent had metacercariæ in the gills; moreover, the metacercariæ in the heart and pericardium outnumbered those in the gills by a ratio of 6:1. In *E. japonicus*, possibly 100 per cent of positive crabs have metacercariæ numbered those in the gills by a ratio of 6:1. In *E. japonicus*, possibly 100 per cent of positive crabs have metacercariæ localized in the gills, while almost no metacercariæ are found in the heart of pericardium.

(6) The inhabitants of the three barrios studied strongly denied eating these crabs in the uncooked condition and sputum and/or fecal examination of 275 people revealed only two (0.7 per cent) positives. Even after allowing for the limitations in efficiency of the technics used in the examinations, a low prevalence rate (in the order of less than 5 per cent) must be accepted for these inhabitants. While this low infection

rate may be accounted for by assuming that a few of the inhabitants do eat the crabs in the raw or inadequately cooked state, accidental infection of man occurring as the result of the preparation of "kinagang" (a native dish for which the juice of the ground-up crab is used) is probably of importance.

(7) The ease with which *Paragonimus* infection was found in domestic cats of the study area, in spite of the very limited number that were examined, strongly indicated that a high percentage of these animals are infected. It is suggested that paragonimiasis in Casiguran may be primarily maintained as an enzootic and that man may only infrequently be involved as a definitive host in the life cycle of the parasite by an accidental mechanism.

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STUDIES ON PARAGONIMIASIS, V *

A SURVEY IN JARO, LEYTE, PHILIPPINES

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TWO PLATES AND ONE TEXT FIGURE

The second of two field investigations made during the course of these studies on paragonimiasis was undertaken by the author in the Municipality of Jaro, Leyte Province, in October, 1955, in order to gather more epidemiologic data about this parasitic disease in the Philippines.

Jaro was chosen on the basis of case reports of paragonimiasis in this country. Of the more than 80 cases mentioned in the literature from 1907 to 1953, 29 had been associated with the province of Leyte by reason of place of birth and/or residence of the infected individuals. Twelve of the 29 cases had been localized to nine municipalities of the province with Jaro having the most—three cases. It was felt, therefore, that chances were excellent for finding persons with paragonimiasis in this locality. This choice was strengthened by information from Dr. T. P. Pesigan, director of the Schistosomiasis Control Pilot Project in Leyte. According to Dr. Pesigan, one of his schistosomiasis teams had found *Paragonimus* ova in the feces of several persons included in a survey of Jaro in 1951.

Other fortunate circumstances made Jaro a good choice. The Bureau of Health, through the Provincial Health Officer of Leyte and the Municipal Health Officer of Jaro, gave the author full cooperation and assistance which included the loan of a jeep for local transportation and the services of a guide and interpreter. The facilities of the schistosomiasis laboratory in Palo, 25 kilometers from Jaro, were offered by Dr. Pesigan. Finally, visits to outlying areas of the municipality could be made because of the excellent peace and order situation prevailing in Jaro.

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THE STUDY AREA

The Island-Province of Leyte is divided into an eastern and western halves by a range of high mountains that cross it from the northwest to the southeast. Nestling at the foothills of the eastern slope of this range is the inland Municipality of Jaro, located about 15 kilometers from Carigara Bay on the north and 25 kilometers from Leyte Gulf on the east. Jaro is 37 kilometers by road from Tacloban City, the provincial capital.

Jaro has a population of more than 26,000, of which less than 7,000 live in the town proper (poblacion); the rest of the population are scattered in 31 smaller, outlying population centers (barrios). These barrios have a population ranging from just over a hundred to almost two thousand, with most of them having about 500 people. No map of the municipality could be obtained, although from the office of the town mayor a sketch map, showing the polling places in the municipality was available—a circumstance indicative of the preoccupation of the people at the time of this survey.

Jaro is almost exclusively agricultural, with coconut (for copra) and rice the most important products. Abaca (for Manila hemp) is grown to a lesser extent. Jaro does not differ much from many other inland Philippine towns located close to the mountains. The country is rough and rolling, with typically tropical vegetation, and is criss-crossed by large and small streams. A river (Cabiyongan R.) runs just south of the poblacion and this river receives tributaries coming from the barrios in the southern half of the municipality. The streams from the barrios in the northern half empty into another river (Minusuang R.). Both rivers open eventually into Carigara Bay.

In the vicinity of Jaro, the rainfall is very heavy and almost uniformly distributed throughout the year. The annual rainfall averages about 120 inches. The heaviest rainfall, 11 to 18 inches per month, occurs during November, December and January, while each of the other months average 7 to 10 inches of rain. There is no dry season and there are about 165 rainy days in each year with about 10 to 19 rainy days in each month.

Barrio Pitogo, where most of the survey work was done, is located about 2 kilometers south of the poblacion. There is

no road to Pitogo, only a winding trail that goes up and down hill and across several streams. The inhabitants of Pitogo usually walk in going to the poblacion, although a family may occasionally travel in a bamboo sled drawn by a carabao (water buffalo). The coconut tree dominates the landscape of Pitogo. Pitogo has an estimated population of 500, although the actual population may be much less. The people are mostly poor farmers and their families. The barrio center is a group of about ten houses, made of bamboo and thatch, located close to each other on both sides of the trail that serves as the main street of the barrio. Other inhabitants live in houses scattered far from each other. Sanitary facilities are totally absent in Pitogo. There are no toilets and the people defecate in the fields. Water for all purposes, including that for drinking, must be obtained from the two streams that are located within a few hundred meters of the barrio center. Most of the people depend on the coconut for their livelihood, processing the coconut into copra by primitive means. Some views of Barrio Pitogo are shown in Plate 1.

Barrio Macanip, which was visited on one occasion, is reached by walking another three kilometers to the south from Pitogo. It has a population of almost 800, and a much more progressive and prosperous air than Pitogo. The houses here are better built and kept. This difference from Pitogo is apparently due to the influence of the teachers in the elementary school in Macanip.

MATERIALS AND METHODS

SURVEY OF STREAMS IN THE STUDY AREA

A survey of streams to note their physical characteristics was made. For some streams, a temperature reading of the water was taken and the pH determined by the use of a LaMotte Standards Comparator.

After the physical inventory, the stream was inspected for the presence of the snail *Brotia asperata* and the crab *Parathelphusa* (*Barythelphusa*) *grapsoides*. A hand was hired to catch some crabs and an on-the-spot examination of the crabs was made to determine infection with *Paragonimus* metacercariæ. A few shrimps from some streams were also examined for infection with the trematode.

STUDY OF THE INTERMEDIATE HOSTS

Aside from a cursory inspection of the streams for the presence of *Brotia asperata,* no other examination involving the snail host was made.

P. (*B.*) *grapsoides* were collected on the spot at daytime during the survey of the streams. In addition, help was hired to collect crabs at night from Masaili stream and Agong-ongan River in Barrio Pitogo. Bamboo traps with coconut meat bait were used in making these night collections.

Collected crabs were examined by either one of two methods:

1. Rapid examination—as described in the report of the Casiguran survey.(9)
2. Detailed examination. The heart and pericardium and the gills were dissected out of the crab and placed in a Petri dish containing water or normal salt solution. Any metacercariæ in these tissues were teased out with dissecting needles and counted under a dissecting microscope (magnification, 10x). The body muscles of the crab were placed in a Petri dish and thoroughly teased. This tissue was then transferred to a beaker, and, by using water, was subjected to repeated sedimentation and decantation until the supernate was clear. The sediment, after the last decantation, was examined under the dissecting microscope and the metacercariæ were pipetted off as they were counted. This method was used in the examination of crabs that were collected from Masaili Stream and Agong-ongan River and brought to the laboratory in Manila.

STUDY OF THE HUMAN POPULATION

The study of the human population involved the following:

a. Sputum and/or feces examination
b. Intradermal test for paragonimiasis
c. Inquiry into eating habits, etc.

The group principally involved in this phase of the survey was the population of Barrio Pitogo. However, this study included the examination of other groups of persons, inclusion of which gave more information either on the ecology of paragonimiasis in Jaro or on the efficiency and limitations of the intradermal test for paragonimiasis.

Sputum and feces examination.—The original plan of study was to skin-test the subjects that could be gathered in one place and to distribute containers for feces and sputum to individuals as they were skin-tested. The specimens could then be collected by a native of the place and brought to the laboratory in Palo for examination. Examination of the sputum, by

direct smear and concentration using 3 per cent sodium hydroxide, and of the feces, by the acid-ether technic(10) as were done in Sorsogon, could then be performed in a laboratory where an electric centrifuge was available. This did not prove to be a practical plan for barrio Pitogo and had to be abandoned, after the first visit to that barrio, in favor of a house-to-house survey. In these house visits, the members of the household, except the very young children, were requested to hawk up sputum. The sputum was examined on the spot, as these persons were being skin-tested. For the most part, therefore, the results of the sputum examination reported in this paper were based on a direct smear of likely-looking portions of a very little sputum sample. All fecal specimens, however, were examined by the acid-ether technic.

Intradermal test for paragonimiasis.—The antigen used for skin-testing subjects for paragonimiasis in this study was given by Dr. Muneo Yokogawa of the Institute of Public Health at Tokyo, Japan, to the author. The description of the antigen, the technic of the test and other pertinent data are given in the presentation of the results and observations.

STUDY ON OTHER DEFINITE HOSTS OF PARAGONIMUS

Five cats were bought from the inhabitants of Barrio Pitogo. These cats were examined in the laboratory at Palo for the presence of *Paragonimus* adults in the lungs at autopsy. Feces from the large intestine of these cats was obtained and examined for *Paragonimus* ova by the acid-ether technic.

RESULTS AND OBSERVATIONS

THE STREAM SURVEY

Sixteen streams, the relative position of which are shown by numbers in a map of Jaro and vicinity (Plate 2), were included in the survey. Streams 1 to 10 were seen by the side or crossing the road leading from Jaro to Carigara on the north; Stream 11 was seen on the road from Jaro to Alang-alang; Streams 12 and 13 are located in Barrio Pitogo and Streams 14 to 16 in Barrio Macanip. Except for Streams 9 and 10, which are situated in Tunga Municipality, all these streams are in the Municipality of Jaro.

1. Cabiyongan River (Plate 3, fig. 1)—located just south of the poblacion running north and northwest, parallel to road. Wide, about 60 ft., 1 to 5 feet deep, low banks. Stony bed with big boulders. No covering vegetation. Flow very swift. At 2:00

FIG. 1. The location of the sixteen streams and rivers surveyed in Jaro and vicinity. In the streams indicated by a circle, *P.* (*B.*) *grapsoides* harboring *Paragonimus* metacercariae were present; no infected *P.* (*B.*) *grapsoides* were found in the streams indicated by a triangle.

p. m., with air temperature at 82° F., water temperature was at 80° F. pH 8.0.

Brotia asperata—none seen.

P. (*B.*) *grapsoides*—none of the 3 positive.

2. Sitio Macupa Creek—1 km. from poblacion. 3 to 4 ft. wide, 1 ft. deep, low banks. Bed of small stones, swiftly flowing, clear water. Partly shaded.

Brotia asperata—many.

P. (*B.*) *grapsoides*—9 of 9 positive.

3. Hibucauan Stream—Barrio Hibucauan, 3 km. from poblacion. 7 to 12 feet wide, 1 to 4 ft. deep. Steep banks, bed of mud and small boulders. Swiftly flowing, slightly muddy water. Partly shaded.

Brotia asperata—none seen.

P. (*B.*) *grapsoides*—9 of 14 positive.

Shrimps—present, negative.

4. Manolbong Creek—in Barrio Hibucauan, 4.6 km. from poblacion. 6 ft. wide, 1 ft. deep. Bed of rocks and stone, banks quite steep. Vegetation covering banks only. Swiftly flowing, clear water. With air temperature at 82° F., water temperature 78° F. pH 7.5.

Brotia asperata—present.

P. (B.) grapsoides—6 of 6 positive.

5. Bongling Creek—at boundary between barrios Hibucauan and Hiagsam, 4.85 km. from poblacion. 4 to 5 ft. wide, 1 ft. deep. Bed stony, low banks. Unshaded. Flow, moderate. Water, clear with a temperature of 78° F. when air temperature was 82° F. pH 7.0.

Brotia asperata—none seen.

P. (*B.*) *grapsoides*—1 of 2 positive.

6. Hiagsam Brook—at Barrio Hiagsam, 6 km. from poblacion. 2 to 3 ft. wide 1 ft. deep. Stony bed, steep banks. Shaded. Flow moderate, clear water.

Brotia asperata—present.

P. (*B.*) *grapsoides*—10 of 22 positive.

7. Kabunga-an Creek—very near to Stream 6, just over 6 km. from poblacion, in Barrio Mohon. 4 ft. wide, 1 ft. deep. Rocky bed, steep banks. Partly shaded. Fast flowing, clear water. With air temperature at 80° F., water temperature, 78° F. pH 7.5.

Brotia asperata—present.

P. (B.) grapsoides—4 of 5 positive.

8. Hubas Stream—in Barrio Mohon, 6.52 km. from poblacion. 4 ft. wide, 1 ft. deep. Stony bed, low banks. Shaded. Swiftly flowing, clear water, temperature, 78° F. pH 7.5.

Brotia asperata—present.

P. (*B.*) *grapsoides*—5 of 5 positive.

9. Tunga River—in the Municipality of Tunga, 7 km. from Jaro poblacion. 40 ft. wide, several ft. deep. Sandy bed, with stones and

rocks, and low banks. No covering vegetation. Very swiftly flowing clear water. With air temperature at 82° F., water temperature, 78° F. pH 8.0.

Brotia asperata—none seen.

P. (*B.*) *grapsoides*—none of 3 positive.

10. Baliri Stream—in the Municipality of Tunga, 8 km. from Jaro poblacion. 15 ft. wide, shallow. Bed of sand and stone, low banks. No covering vegetation. Slow-flowing, clear water, temperature, 78° F. pH 7.5.

Brotia asperata—none seen.

P. (B.) grapsoides—3 of 4 positive.

11. Barrio Galotan Stream—4 km. from Jaro poblacion on road to Alangalang. 3 to 4 ft. wide, 2 ft. deep. Stony bed, steep banks. Partly shaded. Swiftly flowing, clear water, with temperature of 82° F. when air temperature was 84° F. pH 7.5.

Brotia asperata—none seen.

P. (B.) grapsoides—none of 4 positive.

12. Masaili Stream (Plate 3, figs. 2 and 3)—in Barrio Pitogo, about 200 meters north of the barrio center. 3 to 8 ft. wide, 1 ft. deep. Very steep banks, stony bed. Very densely shaded. Swiftly flowing, clear water. At 10:30 a. m. with air temperature at 86° F., water temperature, 78° F. At 8:00 p. m. with air temperature at 80° F., water temperature was still at 78° F. pH 7.5.

Brotia asperata—abundant.

P. (*B.*) *grapsoides*—first collection—28 of 29 positive.

Second collection—34 of 34 positive.

13. Agong-ongan River—in Barrio Pitogo, about 300 meters south of barrio center. 20 to 40 ft. wide, 1 to 3 feet deep. Very stony and rocky bed, very gradually sloping banks. Vegetation shading only the banks. Very swiftly flowing, clear water. At 3.30 p. m. with air temperature at 86° F., water temperature, 80° F., at 8:00 p. m., with air temperature at 80° F., water temperature was 78° F. pH 7.5.

Brotia asperata—none seen.

P. (*B.*) *grapsoides*—first collection—17 of 26 positive.

Second collection—7 of 25 positive.

14. Sariyong Creek—in Barrio Macanip, west of barrio center. 5 ft. wide, 2 ft. deep. Bed of sand and stone, barks gradually sloping. Partly shaded. Swiftly flowing, clear water.

Brotia asperata—none seen.

P. (*B.*) *grapsoides*—73 of 90 positive.

15. Mainit River—in Barrio Macanip, west of barrio center. 20 ft. wide, 2 ft. deep. Bed of sand and stone, low banks. Unshaded. Swiftly flowing, clear water.

Brotia asperata—none seen.

P. (*B.*) *grapsoides*—none of 5 positive.

16. Hagosonis Stream—in Barrio Macanip, west of barrio center. 4 to 6 ft. wide, 1 ft. deep. Rocky bed, steep banks. Shaded.

Swiftly flowing, clear water. At 3.30 p. m. with air temperature at 91° F., water temperature, 82° F. pH 7.5.

Brotia asperata—none seen.

P. (*B.*) *grapsoides*—3 of 3 positive.

INFECTION IN THE CRUSTACEAN HOST

Of 65 *P.* (*B.*) *grapsoides* from Masaili stream, 64 or 98.5 per cent were found positive for *Paragonimus* metacercariæ, while 24 or 47.2 per cent of 51 from Agong-ongan River were found positive. The number of metacercariæ in 36 positive crabs from Masaili stream, as determined by counting these larvæ in the heart and pericardium, gills, and body muscles, varied from 1 to 112, with a median of 12 and a mean of 27. The

TABLE 1.—*Results of the examination of 36 positive crabs, P.* (*B.*) *grapsoides, from Masaili Stream, Barrio Pitogo, for Paragonimus metacercariæ.*

Crab number	Sex	Carapace length by width	Number of *Paragonimus* metacercariæ			
			Total in three tissues	In heart and pericardium	In gills	In body muscles
		mm				
1	F	31 × 27	1	0	0	1
2	F	32 × 28	1	1	0	0
3	M	35 × 31	1	0	0	1
4	M	30 × 26	1	0	0	1
5	M	31 × 27	2	0	0	2
6	F	33 × 28	2	0	0	2
7	F	32 × 28	2	1	0	1
8	F	38 × 33	2	2	0	0
9	F	28 × 23	3	1	0	2
10	F	44 × 38	3	1	0	2
11	M	28 × 24	3	1	0	2
12	M	43 × 36	3	0	0	3
13	F	33 × 27	3	2	0	1
14	M	33 × 28	4	3	0	1
15	F	35 × 30	4	0	0	4
16	M	29 × 24	7	0	0	7
17	M	35 × 29	9	2	0	7
18	F	42 × 34	11	0	1	10
19	M	30 × 25	13	0	0	13
20	F	31 × 26	16	11	0	5
21	F	46 × 40	21	3	1	17
22	M	42 × 35	24	4	0	20
23	M	43 × 36	27	11	1	15
24	M	40 × 34	31	11	2	18
25	F	45 × 39	34	8	2	24
26	M	40 × 34	35	6	2	27
27	F	45 × 37	39	12	13	14
28	M	21 × 18	46	17	0	29
29	M	32 × 25	49	23	5	21
30	F	48 × 41	57	21	5	31
31	F	50 × 40	69	32	17	20
32	M	43 × 36	78	17	7	54
33	F	40 × 34	80	18	8	54
34	F	52 × 44	85	31	12	42
35	F	46 × 39	94	34	7	53
36	M	41 × 32	112	38	10	64
Total			972	311	93	568
Per cent of total by tissue				(32.0)	(9.6)	(58.4)
Number of crabs positive by tissue				26/36	15/36	34/36
Per cent of crabs positive by tissue				(72.2)	(41.7)	(94.4)

number of metacercariæ were counted in only 7 positive crabs from Agong-ongan River, and the counts were 1, 1, 1, 1, 3, 4, and 12. *P.* (*B.*) *grapsoides* from Agong-ongan River not only showed a lower percentage of infection than those from Masaili stream, but also apparently had a lighter metacercarial load.

The variability in the percentage of infection in crabs taken from the same body of water was observed in two collections from Agong-ongan River. Of a collection of 26 crabs examined by the rapid method, 17 or 65 per cent were found positive; of another collection of 25, examined in detail, only 7 or 28 per cent found positive.

Table 1 shows the number and distribution of *Paragonimus* metacercariæ in 36 *P.* (*B.*) *grapsoides* collected from Masaili stream in Pitogo. Metacercariæ were found in the body muscles of 34 (94.4 per cent) of the 36 crabs; in the heart and pericardium of 26 (72.2 per cent); and in the gills of 15 (41.7 per cent). Of the total 972 metacercariæ counted in these three situations, 568 (58.4 per cent) were in the body muscles; 311 (32.0 per cent) were in the heart and pericardium; and 93 (9.6 per cent) were in the gills.

TABLE 2.—*Metacercarial load according to sex and size of the carapace of 36 positive P.* (*B.*) *grapsoides.*

Maximum length of carapace in mm	Male		Female	
	Number examined	Number of *Paragonimus* metacercariæ	Number examined	Number of *Paragonimus* metacercariæ
21 to 30	5	-1-3-7-13-46-	1	-3-
31 to 40	7	-1-2-4-9-31-35-49-	9	-1-1-2-2-2-3-4-16-80-
41 to 50	5	-3-24-27-78-112-	8	-3-11-21-34-39-57-69-94-
51	0		1	-85-

In Table 2 is given the metacercarial load in the 36 positive *P.* (*B.*) *grapsoides*, according to sex and maximum length of the carapace. In this small sample, no difference in metacercarial load between the sexes is apparent, but there is an indication that the metacercarial load is heavier in the larger (older) crabs than in the smaller (younger) ones.

THE HUMAN POPULATION

Eating habits pertinent to the transmission of paragonimiasis.—Like many places in the Philippines which are relatively distant

from the coast, fresh fish and other food from the sea are difficult to obtain in Jaro, and then only at high prices. As a result, many of the residents of the municipality, especially the poorer people such as those of Barrio Pitogo, depend a great deal upon what food may be gotten from the rivers and streams in their vicinity. Shrimps and snails are obtained from these waters as are crabs, which are caught by hand or by the use of bamboo traps. These river crabs are therefore a common food item of the people.

In this locality, crabs are eaten after boiling, or as "sinugba," or as "kinilao." Crabs, boiled or as "sinubga," are eaten by everyone. The word "sinugba" means roasted. The crab is simply roasted over the glowing embers of wood charcoal. Not as many people eat the crab as "kinilao," although a very considerable portion of the population like this dish. For example, about 40 per cent of the people of Barrio Pitogo admitted the eating of crab "kinilao."

As "kinilao," the crab is eaten in the frankly raw state. To prepare this dish, the sour juice from a tiny local variety of citrus fruit "calamansi" and the milk from grated coconut "gata" are merely added to the edible portions of the raw crab. While a "kinilao" of crabs is also eaten at other occasions in Barrio Pitogo, it is a great favorite with the adults during the local "cocktail" hour when these people are downing "tuba"—the local alcoholic drink which is the fermented sap from the coconut tree.

Sputum and/or feces examination for Paragonimus ova.—Of 94 residents of Barrio Pitogo who were skin-tested for paragonimiasis, 83 submitted sputum and/or fecal specimens. Twelve of the 83 submitted both sputum and fecal specimens, 62 submitted sputum only, and nine, feces only.

Paragonimus ova were found in the specimens from 4 (4.8 per cent) of the 83. The four positive individuals were all adult males; each had a history of hemoptysis and admitted the eating of crab "kinilao." The sputum specimens from all four showed gross evidence of blood and had great numbers of ova. Stool specimens from three of the four were also positive for the ova, although in very small numbers. No fecal examination was performed for the fourth positive case.

Fourteen adults from Barrio Macanip submitted both sputum and fecal specimens for examination, while one adult submitted

only a sputum specimen. No *Paragonimus* ova were seen in the specimens from these persons.

The intradermal test for paragonimiasis.—The intradermal test for paragonimiasis was used for the first time in the Philippines in this study. In these tests, the recommendations of those who worked on this diagnostic method in Japan, especially those of Dr. Muneo Yokogawa and his group, have been closely followed in the performance, reading and interpretation.

Antigen: The saline extract of adult *P. westermani* in a dilution of 1:10,000. Merthiolate was added to a concentration of 1:10,000.

Control solution: Physiological salt solution, to which had been added merthiolate to the same concentration as in the antigen.

Injections: Intradermal, at the upper half of the volar surface of the forearm. Enough of the antigen was injected to produce a wheal about 4 mm by 4 mm. Another wheal of about the same size was produced about two inches from the other by an injection of the control solution.

Readings: The length and width of the two wheals were measured with a vernier caliper at the following times: right after injection, at 10, and at 15 minutes.

The size of the wheal was read as a single whole number, taking the average of the length and width. Thus, a wheal 4 mm × 4 mm is read as 4 mm; one that is 6 mm by 8 mm is read as 7 mm; a wheal 6 mm × 7 mm is read as 7 mm, etc. (In the tables given in this paper, A_0, A_1, and A_2 are the readings of the antigen wheal at 0, 10, and 15 minutes, respectively; while C_0, C_1, and C_2 are the readings of the control wheal at the same time intervals.)

Interpretation: The interpretation of the test is based on the enlargement of the antigen wheal in excess of any enlargement of the control wheal. Thus,

Enlargement = (A_2 or A_1 minus A_0) minus (C_2 or C_1 minus C_0)

Whichever is larger between A_2 and A_1 and between C_2 and C_1 is used in the formula.

Negative reaction: no enlargement or an enlargement up to 3 mm.

Doubtful reaction: an enlargement of 4 mm.

Positive reaction: an enlargement of 5 mm or more.

IN NINETY-SIX HEALTHY SUBJECTS

In order to study the response of Filipinos free of the disease to the intradermal test for paragonimiasis, 96 medical and hygiene students of the University of the Philippines were tested. There were 67 males and 29 females in this group, whose ages varied from 17 to 29 years with the average somewhere in the early twenties. These students came from different parts of the country. At first, it was thought to limit this control group only to those students who were positive that they had never

eaten raw or half-cooked crabs, and to eliminate 20 of the 96 who admitted having eaten such food or who could not be sure that they had not. However, since these 20 did not react to the intradermal test differently from the rest, they are included in this group of healthy subjects.

Table 3 summarizes the reaction of these students to the intradermal test for paragonimiasis. There was no increase in size of the antigen wheal in 33.4 per cent of the students, but there was an increase of 1 mm in 49.0 per cent of 2 mm in 10.4 per cent, of 3 mm in 6.2 per cent, and of 4 mm in 1.0 per cent. The control wheal did not enlarge in 57.3 per cent, but there was an increase of 1 mm in 28.1 per cent, of 2 mm in 11.5 per cent, of 3 mm in 2.1 per cent, and of 4 mm in 1.0 per cent. In considering the enlargement of the antigen wheal beyond that of the control wheal, however, it was found that the antigen wheal did not enlarge beyond that of the control wheal in 60 (62.5 per cent) of the 96 subjects, it enlarged by 1 mm in 31 (32.3 per cent), and by 2 mm in 5 (5.2 per cent). According to the interpretation of the test, therefore, all 96 healthy subjects reacted negatively to the intradermal test for paragonimiasis.

IN NINETY-FOUR INHABITANTS OF BARRIO PITOGO

A total of 94 inhabitants of the endemic barrio of Pitogo were tested intradermally for paragonimiasis. There were 58 male subjects and 36 females in this group and their ages varied from 1 year to 76 years. A summary of the results of the skin test in these subjects, together with information on a history of hemoptysis and the eating of crab "kinilao," broken down according to age and sex, is presented in Table 4.

Thirty-one (33 per cent) of these people reacted positively, 44 (47 per cent) had a negative reaction, and 19 (20 per cent), a doubtful reaction. Some positive reactions to the skin test are shown in Plate 4, figs. 1 and 2, and in Plate 5, fig. 1.

Of the 94 persons examined in Barrio Pitogo, 18 (19 per cent) gave a history of hemoptysis, while 76 denied such history. Of the 18 with history of hemoptysis, 10 (56 per cent) reacted positively to the intradermal test; 4 (22 per cent) had a negative reaction; and 4 (22 per cent) showed a doubtful response. Of the 76 who had no history of hemoptysis, 21 (28 per cent) showed a positive response to the test; 40 (52 per cent) reacted negatively; and 15 (20 per cent) had a doubtful response. The percentage of positive reactors is significantly higher in the

TABLE 3.—*Results of intradermal test for paragonimiasis in 96 medical and hygiene students.*

	Number examined	Enlargement of antigen wheal					Enlargement of control wheal					Enlargement of antigen wheal beyond control wheal		
		0	1	2	3	4	0	1	2	3	4	0	1	2
		mm	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*
Males	67	23	28	10	5	1	38	16	10	2	1	41	22	4
Per cent		(34.3)	(41.7)	(15.0)	(7.5)	(1.5)	(56.7)	(23.9)	(14.9)	(3.0)	(1.5)	(61.2)	(32.8)	(6.0)
Females	29	9	19	0	1	0	17	11	1	0	0	19	9	1
Per cent		(31.0)	(65.5)	(0.0)	(3.5)	(0.0)	(58.5)	(38.0)	(3.5)	(0.0)	(0.0)	(65.5)	(31.0)	(3.5)
With no history of eating raw crab	76	28	37	7	3	1	46	22	6	1	1	48	25	3
Per cent		(36.9)	(48.7)	(9.2)	(3.9)	(1.3)	(60.5)	(29.0)	(7.9)	(1.3)	(1.3)	(63.2)	(32.9)	(3.9)
With positive or doubtful history	20	4	10	3	3	0	9	5	5	1	0	12	6	2
Per cent		(20.0)	(50.0)	(15.0)	(15.0)	(0.0)	(45.0)	(25.0)	(25.0)	(5.0)	(0.0)	(60.0)	(30.0)	(10.0)
Total	96	32	47	10	6	1	55	27	11	2	1	60	31	5
Per cent		(33.4)	(49.0)	(10.4)	(6.2)	(1.0)	(57.3)	(28.1)	(11.5)	(2.1)	(1.0)	(62.5)	(32.3)	(5.2)

TABLE 4.—*Hemoptysis, the eating of "kinilao" and the results of intradermal test for paragonimiasis in 94 inhabitants of Barrio Pitogo, Jaro, according to age and sex.*

Age group (Years)	Males								Females								Total							
	Hemoptysis		Eating of "kinilao"			Skin test			Hemoptysis		Eating of "kinilao"			Skin test			Hemoptysis		Eating of "kinilao"			Skin test		
	+	(—)	+	(—)	?	+	(—)	D	+	(—)	+	(—)	?	+	(—)	D	+	(—)	+	(—)	?	+	(—)	D
0–4	0	6	0	5	1	0	6	0	0	4	0	4	0	0	4	0	0	10	0	9	1	0	10	0
5–9	0	10	1	9	0	4	4	2	0	4	0	4	0	1	2	1	0	14	1	13	0	5	6	3
10–14	1	4	2	3	0	2	3	0	0	1	0	1	0	0	1	0	1	5	2	4	0	2	4	0
15–19	0	3	2	1	0	3	0	0	0	4	1	2	1	2	2	0	0	7	3	3	1	5	2	0
20–29	5	14	16	3	0	8	5	6	3	8	2	9	0	3	5	3	8	22	18	12	0	11	10	9
30–39	5	5	6	4	0	3	6	1	0	7	1	4	2	2	4	1	5	12	7	8	2	5	10	2
40–49	1	0	1	0	0	1	0	0	1	1	1	1	0	0	0	2	2	1	2	1	0	1	0	2
50+	1	3	2	2	0	1	2	1	1	2	2	1	0	1	0	2	2	5	4	3	0	2	2	3
Total	13	45	30	27	1	22	26	10	5	31	7	26	3	9	18	9	18	76	37	53	4	31	44	19
Per cent	22	78	52	46	2	38	45	17	14	86	19	72	9	25	50	25	19	81	39	56	5	33	47	20

+, Positive; (—), Negative; ? or D, Doubtful

group with a history of hemoptysis than in the group without such a history.

Of the 94, 37 (39 per cent) admitted having eaten crabs prepared as "kinilao," while the rest denied having eaten this dish. Of the 37, 19 (51 per cent) showed a positive reaction to the skin test; 8 (22 per cent) reacted negatively; and 10 (27 per cent) showed a doubtful response. Of the 57 who denied eating the raw crab dish, only 12 (21 per cent) reacted positively; 35 (61 per cent) had a negative reaction; and 10 (18 per cent) showed a doubtful response. The percentage of positive reactors is significantly higher in the group which admitted eating the raw crab dish than in the group which denied eating it.

Of the 58 males included in this survey, 22 (38 per cent) gave a positive reaction to the intradermal test; 26 (45 per cent) showed a negative reaction; and 10 (17 per cent) responded doubtfully. Of the 36 females, 9 (25 per cent) showed a positive reaction; 18 (50 per cent) reacted negatively; in 9 (25 per cent), the reaction was doubtful. While the percentage of positive reactions is higher among the males than the females, the difference is not statistically significant. There are two few persons in this sample for a good comparison of the reaction to the intradermal test for paragonimiasis by age group; however, it is noteworthy that no child in the age group 0 to 4 years reacted positively.

IN INHABITANTS OF BARRIO MACANIP

Eighteen adults from Barrio Macanip were tested intradermally for paragonimiasis. The reaction was positive in 5, negative in 9, and doubtful in 4.

IN TWELVE DEFINITE CASES OF PARAGONIMIASIS

The reactions to the intradermal test for paragonimiasis of twelve individuals who were positively known to be infected with *Paragonimus* are shown in Table 5.

Subjects 1 to 4 of this table were the individuals from Pitogo who were found positive for *Paragonimus* ova in the sputum.

Upon the arrival of the author in Leyte, he was very kindly furnished by Dr. T. P. Pesigan with a list of 8 persons from Jaro who had been found positive for *Paragonimus* ova in the feces during a schistosomiasis survey in 1951. Contact with three of these persons was made and they are listed as subjects 6, 7, and 8 in Table 5. Subjects 6 and 7 were found to be still

TABLE 5.—*Intradermal test for paragonimiasis in 12 definite cases of paragonimiasis.*

Subject number	Initial	Sex	Age (Years)	Intradermal test for paragonimiasis						Enlargement [b]	Interpretation [b]
				Reading of antigen wheal (in mm)			Reading of control wheal (in mm)				
				0 min (A_0)	10 min (A_1)	15 min (A_2)	0 min (C_0)	10 min (C_1)	10 min (C_2)		
1	H. E.	M	26	6 × 6 (6)	10 × 11 (11)	10 × 11 (11)	6 × 6 (6)	6 × 6 (6)	6 × 6 (6)	[a] 5	+
2	P. L.	M	20	5 × 6 (6)	10 × 11 (11)	10 × 11 (11)	6 × 6 (6)	6 × 6 (6)	6 × 6 (6)	5	+
3	M. N.	M	44	6 × 6 (6)	10 × 11 (11)	10 × 12 (11)	6 × 6 (6)	5 × 5 (5)	5 × 5 (5)	[a] 5	+
4	F. G.	M	34	6 × 6 (6)	10 × 10 (10)	10 × 10 (10)	5 × 5 (5)	5 × 5 (5)	4 × 4 (4)	4	+
5	C. C.	M	55	5 × 6 (6)	9 × 9 (9)	9 × 9 (9)	5 × 5 (5)	5 × 5 (5)	5 × 5 (5)	3	D
6	A. C.	F	15	4 × 4 (4)	10 × 10 (10)	11 × 11 (11)	3 × 3 (3)	3 × 3 (3)	3 × 3 (3)	7	+
7	G. C.	M	18	5 × 5 (5)	7 × 8 (8)	7 × 8 (8)	5 × 5 (5)	5 × 5 (5)	4 × 4 (4)	3	+
8	R. C.	F	14	5 × 5 (5)	9 × 9 (9)	9 × 10 (10)	4 × 4 (4)	fading	fading	5	+
9	A. A.	M	37	6 × 6 (6)	10 × 20 (15)	10 × 20 (15)	4 × 5 (5)	3 × 3 (3)	fading	[a] 9	+
10	G. H.	M	44	6 × 7 (7)	11 × 13 (12)	12 × 13 (13)	7 × 9 (8)	7 × 9 (8)	7 × 9 (8)	[a] 6	+
11	A. K.	M	17	4 × 4 (4)	7 × 10 (9)	8 × 10 (9)	5 × 6 (6)	5 × 6 (6)	fading	[a] 5	+
12	C. M.	F	10	5 × 6 (6)	12 × 13 (13)	13 × 14 (14)	3 × 3 (3)	5 × 5 (5)	5 × 5 (5)	[a] 6	+

+, Positive; (—), Negative; D, Doubtful.

[a] Indicates an amoeboid reaction.

[b] Please consult text for explanation.

passing out *Paragonimus* ova in the sputum at the time of the skin test but subject 8 did not submit sputum or fecal specimens for examination. Subject 6 came for the intradermal test accompanied by her father, who was at the time having blood streaks in his sputum. Examination of the sputum of this individual showed *Paragonimus* ova and he is included in this table as subject 5.

Subjects 9 and 10 were old cases of paragonimiasis diagnosed in the Leyte Provincial Hospital and in the Tacloban Tuberculosis Pavilion.(8) Subjects 11 and 12 were ward cases in the San Lazaro Hospital in Manila whose sputum specimens were positive for *Paragonimus* ova.

Nine (75 per cent) of the twelve reacted positively to the intradermal test for paragonimiasis; two showed a negative response while one had a doubtful reaction.

IN TWENTY INDIVIDUALS WITH SCHISTOSOMIASIS JAPONICA

The areas of endemicity of schistosomiasis japonica and paragonimiasis overlap in some parts of the Philippines. It is therefore important to find out whether schistosomiasis will cause a cross reaction with the intradermal test for paragonimiasis and give rise to false positives. A chance to investigate this point presented itself during the author's stay in Palo, Leyte in the last few days of this survey trip. With the generous help of the personnel of the Schistosomiasis Control Division, 20 persons definitely known to have schistosomiasis were studied. Each schistosomiasis patient was given three intradermal injections: *Paragonimus* antigen, control solution, and *Schistosoma* antigen. The results of these tests are given in Table 6.

Not one of the 20 schistosomiasis cases gave a positive reaction to the intradermal test for paragonimiasis. To the intradermal test for schistosomiasis, according to the reading and interpretation of the test as given by Pesigan, et al.,(2) 7 of the 20 reacted positively; 8 had doubtful reactions, and 5 showed a negative response. Plate 5, fig. 2 illustrates the reaction of Subject 7 in Table 6 to the two intradermal tests.

IN THIRTY-SIX PERSONS WITH CONFIRMED OR PROBABLE TUBERCULOSIS

What effect, if any, does tuberculosis have on the intradermal test for paragonimiasis? This will be an important consideration in any screening test for paragonimiasis in the Philippines, since tuberculosis is so prevalent that any population group

TABLE 6.—*Intradermal tests for paragonimiasis and schistosomiasis in 20 subjects with schistosomiasis japonica.*

Subject number	Initial	Sex	Age (Years)	Intradermal test for paragonimiasis							Intradermal test for schistosomiasis				
				Reading of antigen wheal (in mm)			Reading of control wheal (in mm)			Enlargement	Interpretation [a]	Reading of antigen wheal (in mm)			Enlargement	Interpretation [b]
				0 min	10 min	15 min	0 min	10 min	15 min	30 min			0 min	15 min	30 min		
1	M. B.	M	46	5 5 5 5 6 6	5 5 6 7 7 7	5 5 5 6 6 7	5 5 5 5 5 6	5 4 5 5 5 6	6 4 5 5 5 6	6 4 fading 5 5 6	0	(−)	6 5 5 5 5 6	12×15 5×5 8×9 11×13 6×7 7×9	12×15 5×5 10×10 11×13 6×7 7×10	7	+
2	P. R.	F	9	5 5 5 5 5 5	5 5 5 6 6 7	5 5 5 6 6 7	5 5 5 5 5 6	5 4 5 5 5 6	6 4 5 5 5 6	6 4 fading 5 5 6	0	(−)	6 5 5 5 5 6	12×15 5×5 8×9 11×13 6×7 7×9	12×15 5×5 10×10 11×13 6×7 7×10	0	+
3	T. B.	F	19	5 5 5 5 5 5	5 5 5 6 6 7	5 5 5 6 6 7	5 5 5 5 5 6	5 4 5 5 5 6	6 4 5 5 5 6	6 4 fading 5 5 6	0	(−)	6 5 5 5 5 6	12×15 5×5 8×9 11×13 6×7 7×9	12×15 5×5 10×10 11×13 6×7 7×10	5	+
4	C. F.	F	45	3 4 5 5 6 6	4 5 6 6 7 7	5 5 6 6 7 7	5 5 5 5 5 6	5 4 5 5 5 6	6 4 5 5 5 6	6 4 fading 5 5 6	0	(−)	6 5 5 5 5 6	12×15 5×5 8×9 11×13 6×7 7×9	12×15 5×5 10×10 11×13 6×7 7×10	6	+
5	S. L.	F	9	5 5 5 5 6 6	5 5 5 6 6 7	5 5 5 6 6 7	5 5 5 5 5 6	5 4 5 5 5 6	6 4 5 5 5 6	6 4 fading 5 5 6	1	(−)	6 5 5 5 5 6	12×15 5×5 8×9 11×13 6×7 7×9	12×15 5×5 10×10 11×13 6×7 7×10	2	+
6	B. B.	M	13	5 5 5 5 6 6	5 5 6 6 6 7	5 5 5 6 6 7	5 5 5 6 6 6	5 4 5 5 5 6	5 4 5 5 5 6	5 4 fading 5 5 6	1	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	4	+
7	G. T.	F	7	4 4 5 5 6 6	4 5 5 6 6 7	5 5 5 6 6 7	4 4 5 5 5 6	3 4 5 5 5 6	3 4 5 5 5 6	3 4 fading 5 5 6	0	(−)	4 4 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	0	+
8	R. C.	M	8	4 4 4 4 5 5	4 4 5 5 6 6	4 4 5 5 6 6	4 4 4 4 5 5	3 4 4 4 5 5	4 4 5 5 5 6	4 4 fading 5 5 6	1	(−)	4 4 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	0	+
9	F. S.	M	8	4 4 5 5 5 6	4 4 5 5 6 6	4 4 5 5 6 6	4 4 5 5 5 6	4 4 5 5 5 6	5 4 5 5 5 6	5 4 fading 5 5 6	0	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	2	+
10	S. N.	F	8	5 5 5 5 6 6	5 5 5 6 6 7	5 5 5 6 6 7	5 5 5 5 5 6	5 4 5 5 5 6	5 4 5 5 5 6	5 4 fading 5 5 6	0	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	2	+
11	M. A.	F	7	4 5 5 5 6 6	4 5 5 6 6 7	4 5 5 6 6 7	3 5 5 5 5 6	4 5 5 5 5 6	4 5 5 5 5 6	4 5 fading 5 5 6	0	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	1	+
12	L. M.	F	10	5 5 5 5 5 5	5 5 5 6 6 7	5 5 5 6 6 7	4 5 5 5 5 6	5 4 4 4 4 5	5 4 4 4 4 5	5 4 fading 4 4 5	1	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	4	+
13	F. N.	M	8	5 5 5 5 5 5	5 5 5 6 6 7	5 5 5 6 6 7	5 5 5 5 5 6	5 4 5 5 5 6	5 4 5 5 5 6	5 4 fading 5 5 6	2	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	1	+
14	B. L.	M	9	4 4 5 5 5 6	4 5 5 6 6 7	4 5 5 6 6 7	5 5 5 5 5 6	5 5 5 5 5 6	5 5 5 5 5 6	5 5 fading 5 5 6	0	(−)	4 4 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	4	+
15	C. S.	M	13	5 5 5 5 5 6	5 5 5 6 6 7	5 5 5 6 6 7	5 5 5 5 5 6	5 5 5 5 5 6	5 5 5 5 5 6	5 5 fading 5 5 6	0	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	0	+
16	F. A.	M	29	6 5 5 6 6 7	5 6 6 7 7 7	5 6 6 7 7 7	5 5 5 5 5 6	5 6 6 6 6 7	5 6 6 6 6 7	5 5 fading 5 5 6	0	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	5	+
17	E. M.	F	9	5 5 5 5 6 6	5 6 6 7 7 7	5 6 6 7 7 7	4 5 5 5 5 6	4 5 5 5 5 6	5 5 5 5 5 6	5 5 fading 5 5 6	1	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	3	+
18	W. K.	M	9	5 5 5 5 6 6	5 5 6 6 7 7	5 5 6 6 7 7	5 5 5 5 5 6	4 5 5 5 5 6	5 5 5 5 5 6	5 5 fading 5 5 6	2	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	1	+
19	R. B.	F	27	5 5 5 5 6 6	5 6 6 7 7 7	5 6 6 7 7 7	5 5 5 5 5 6	4 5 5 5 5 6	5 5 5 5 5 6	5 5 fading 5 5 6	3	(−)	5 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×11 4×5 5×5 7×8 6×7 7×10	1	+
20	D. R.	M	15	5 5 5 5 6 6	5 7 7 7 7 8	5 7 7 7 7 8	5 5 5 5 5 6	5 5 5 5 5 6	5 5 5 5 5 6	5 5 fading 5 5 6	3	(−)	4 5 5 5 5 6	8×10 4×5 5×5 6×7 6×6 7×9	9×13 4×5 5×5 7×8 6×7 7×10	6	+

+, Positive; (−), Negative; D, Doubtful.

[a] Please consult text for explanation.

[b] Using the criteria given by Pesigan, et al. (2)

surveyed will most probably include a number of individuals with tuberculosis. Furthermore, the test may be of use in the differential diagnosis between pulmonary tuberculosis and pulmonary paragonimiasis, or in deciding whether the diseases are co-existent in an individual.

To obtain data on whether or not tuberculosis by itself would give rise to a positive reaction to the intradermal test for paragonimiasis, 36 patients, consisting of 19 ward cases and 17 out-patients, of the Tacloban Tuberculosis Pavilion were skin-tested. Tuberculosis was a confirmed diagnosis in most of these cases. The results are given in Table 7. None of the 36 showed a positive reaction to the test, although one showed a doubtful response.

TABLE 7.—*Intradermal test for paragonimiasis in 36 patients with confirmed or probable tuberculosis.*

Subject number	Initial	Sex	Age (Years)	Hemoptysis	Diagnosis	I. T. P.[a]	
						Enlargement	Interpretation
					Ward patients	*mm*	
1	R. R.	F	25	(—)	PTb., Tb. of spine with psoas abscess	3	(—)
2	A. P.	F	55	+	PTb.	0	(—)
3	A. F.	F	20	+	PTb., mod. adv., rt.	1	(—)
4	C. V.	F	19	(—)	PTb. (acid-fast positive)	1	(—)
5	S. P.	F	26	(—)	------do------	0	(—)
6	E. J.	M	22	+	PTb., mod. adv., rt.	0	(—)
7	J. S.	F	76	+	PTb., adv., rt.	0	(—)
8	C. K.	F	28	+	PTb., far adv., rt.	1	(—)
9	A. E.	F	60	+	PTb., far adv., bil.	1	(—)
10	S. A.	F	21	(—)	------do------	1	(—)
11	L. S.	M	38	(—)	------do------	0	(—)
12	C. E.	M	54	(—)	PTb., far adv., bil. (acid-fast positive)	2	(—)
13	A. P.	M	35	------	PTb. (?)	0	(—)
14	R. O.	M	18	(—)	Ptb., far adv., bil.	2	(—)
15	L. J.	M	41	(—)	Ptb., far adv., bil., active (acid-fast positive)	1	(—)
16	P. D.	M	53	(—)	PTb., mod. adv., left (acid-fast positive)	4	D
17	I. S.	M	55	(—)	PTb., mod. adv.	0	(—)
18	J. V.	F	49	+	PTb. c pleurisy and effusion (acid-fast positive)	2	(—)
19	F. J.	M	52	(—)	PTb., far adv., active, bil.	1	(—)
					Out patients		
20	M. M.	F	39	+	PTb., mod. adv., active, bil.	1	(—)
21	E. Q.	F	50	+	------do------	2	(—)
22	R. G.	M	15	+	For observation	0	(—)
23	F. B.	M	39	(—)	PTb., minimal, bil.	1	(—)
24	T. C.	F	50	------	PTb., mod. adv., bil.	0	(—)
25	P. D.	M	54	+	For observation	0	(—)
26	F. B.	M	7	(—)	PTb.	0	(—)
27	B. A.	M	33	(—)	For observation	1	(—)
28	R. R.	M	6	(—)	------do------	0	(—)
29	F. A.	M	37	------	------do------	0	(—)
30	M. B.	F	30	(—)	PTb., mod. adv., active, bil.	3	(—)
31	C. F.	F	25	(—)	PTb., minimal, active, bil.	0	(—)
32	M. D.	M	26	(—)	PTb., fibroid	1	(—)
33	T. M.	F	19	(—)	PTb., (?). For observation	1	(—)
34	F. C.	F	70	(—)	PTb., minimal, bil.	0	(—)
35	M. B.	F	16	(—)	PTb.	2	(—)
36	A. A.	M	34	(—)	Pleurisy with effusion	1	(—)

[a] I.T.P., Intradermal test for paragonimiasis.
(—), Negative; D, Doubtful.

A DEFINITIVE HOST OTHER THAN MAN

Because domestic cats were found infected with *Paragonimus* in the survey in Casiguran, Sorsogon, some cats from Barrio Pitogo were autopsied and examined for infection with the trematode. Four of five cats showed lesions in the lungs due to *Paragonimus*, and *Paragonimus* ova were found in the feces of these positive cats. Two of the cats were very lightly infected, with only once cyst each in the lungs. A third cat had three cysts while the fourth one was heavily infected, with twenty cysts in its lungs.

DISCUSSION

While Jaro is located much farther from the sea coast than Casiguran, the barrios that were studied in these two municipalities resemble each other in terrain and climatic conditions. Both Barrio Pitogo and Barrio Macanip are in hilly terrain and the coconut tree dominates the landscape. A definite dry season, as it is known in the western part of the Philippines, does not occur in the eastern half of Leyte, including Jaro; and the amount of rainfall is almost as heavy as in Casiguran.

The social and economic conditions of the people in Barrio Pitogo differ but little from that of the people in the barrios of Casiguran. Here, in the most primitive rural setting, these poor and uneducated folk live their lives, just getting enough food to keep starvation one step away and raising children who most probably will grow up to live a life as difficult as their parents'.

The impact of education on rural life was well seen in Barrio Macanip. Because of the presence of an elementary school and school teachers in this barrio, there was an atmosphere of struggle for a better way of life—so different from the air of resignation that one could sense in Pitogo. Not only were the people of Barrio Macanip organized to plant vegetables that would improve the variety of their food but even their water supply had received some attention. In this barrio, the people were drawing water for drinking from one spring source and not just from any part of the creek nearby as is the practice with the people of Barrio Pitogo.

ON THE STREAM SURVEY

The survey of the streams showed that *Paragonimus* is able to maintain itself over a wide area of Jaro. In all sixteen streams studied, fresh-water crabs which appeared identical

with *P.* (*B.*) *grapsoides* were present, including streams whose width and other physical characteristics did not conform with our idea of the most favorable natural habitat for this crab species. Of fourteen streams which are found within the geographical limits of Jaro Municipality, *P.* (*B.*) *grapsoides* with *Paragonimus* metacercariæ were found in all but three.

In several streams where infected crabs were obtained, no *Brotia asperata* were found by cursory search. It is probable that these snails were present but not in enough numbers to be easily found, although the possibility that some other species of snail may also serve as the first intermediate host of *Paragonimus* in this locality should not be discounted. The streams where the infection in crabs seemed to be highest were the small, shaded creeks, with steep banks, a stony bed and clear, fast flowing water.

A few words may be said about the pH and temperature of the water in these streams. In Masaili Stream, for example, where *B. asperata* was found and where the crabs were not only numerous but had a high infection rate, the water tended to be on the slightly alkaline side (pH 7.5) and remained cool despite the hot sun, staying at a uniform 78°F while the air temperature changed from 86°F at day time to 80°F in the early night.

ON THE CRUSTACEAN HOST

With the inhabitants of Barrio Pitogo as the focus of attention in this survey, it became important to study *Paragonimus* infection in *P.* (*B.*) *grapsoides* from the two bodies of water in this barrio so that the infective potential to the definitive host could be gauged thereby.

P. (*B.*) *grapsoides* could be obtained in abundance from both Masaili Stream and Agong-ongan River. The significantly higher infection rate and the apparently heavier metacercarial load of the crabs from the former, however, make it clear that the infective potential to the definitive host is greater in crabs obtained from Masaili Stream than in those gathered from Agong-ongan River. Such a situation may be expected in view of the fact that Masaili Stream is very much narrower than Agong-ongan River; therefore, *Paragonimus* cercariæ in Masaili Stream would have a much greater probability of coming in contact with and infecting the crabs.

It is interesting to note that while the infection rate in *P.* (*B.*) *grapsoides* from Masaili Stream was close to 100 per

cent, no positive crabs from this stream was found to have as heavy a metacercarial load as was observed in some crabs from Casiguran. While the relatively early death of very heavily infected crabs may be considered in explaining this observation, the fact that such heavily infected crabs were found in the survey in Casiguran argues against such assumption. The likely explanation appears to be that the people in Pitogo go crab-collecting for food so frequently and regularly that the crabs are not allowed to stay in the stream long enough to build up a metacercarial load as heavy as that observed in Casiguran.

The data that have been presented on the percentage of infection and metacercarial load of crabs from Masaili Stream in Barrio Pitogo justify the conclusion that any person in the habit of eating these crabs in the raw or inadequately cooked condition will, in all probability, become infected with *Paragonimus*. The same statement may be made for persons eating crabs obtained from Agong-ongan River, although the risk of infection in this instance will be considerably less. In reality, however, there is no great reason for considering separately the potential of infection to the definitive host of crabs from the two streams because the inhabitants of Barrio Pitogo obtain crabs for food from both streams throughout the year.

Statistical treatment of the data obtained on the localization of *Paragonimus* metacercariæ in *P.* (*B.*) *grapsoides* from Masaili Stream (Table 2) shows that a significantly higher proportion of infected crabs have metacercariæ in the body muscles than in the heart and pericardium, and in the heart and pericardium than in the gills; and that a significantly greater proportion of metacercariæ are found in the body muscles than in the heart and pericardium, and in the heart and pericardium than in the gills. The localization of *Paragonimus* metacercariæ in *P.* (*B.*) *grapsoides,* as observed in this and the Casiguran surveys, is compared with that in *Eriocheir japonicus* in Table 8, using data given by Yokogawa(5) and Takahashi.(4)

The marked contrast in localization of *Paragonimus* metacercariæ in the heart and pericardium on one hand and in the gills on the other between *P.* (*B.*) *grapsoides* and *E. japonicus* has been brought out in the report of the Casiguran survey,(9) and the data from this second survey give added proof to the existence of such difference. The fact that metacercariæ localize very frequently in the heart and pericardium of *P.* (*B.*)

TABLE 8.—*Comparison of localization of Paragonimus metacercariæ in P. (B.) grapsoides and japonicus.*

Species from different places	Positive crabs	Positive in gills		Positive in heart and pericardium		Positive in body muscles		Metacercarium in three location	Location in gills		Location in heart and pericardium		Location in body muscle	
	Number	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
P. (B.) grapsoides:														
Casiguran, Sorsogon(1)	41	20	48.8	31	75.6	23	68.3	1,881	200	10.6	1,243	66.1	438	23.3
Jaro, Leyte	36	15	41.7	26	72.2	34	94.4	972	93	9.6	311	32.0	568	58.4
E. japonicum:														
Shizuoka Prefecture(5)	75	75	100.0	0	0.0	53	70.6	3,633	2,995	82.4	0	0.0	638	17.6
Ehime Prefecture(6)	41	41	100.0	1	2.4	12	29.3	620	541	87.2	1	0.2	78	12.6

grapsoides may be used as a basis for gross, rapid examination of these crabs in surveys to determine areas in the Philippines where *Paragonimus* is able to maintain itself. The difference in behavior of the parasite in the two crab species may also be utilized as a tool for the investigation of the fundamental problem of host-parasite relationships.

Some inconsistency of observations in Table 8 is evident in the localization of *Paragonimus* metacercariæ in the body muscles of the crab. In so far as the observations in *P.* (*B.*) *grapsoides* are concerned, the inconsistency is explanable on the basis of the difference in technics used in the examination of the body muscles for metacercariæ in the two surveys. In the Sorsogon survey, the body muscles were examined as a press preparation between two slides and this technic is less efficient than the one used in this second survey. The localization of *Paragonimus* metacercariæ in *P.* (*B.*) *grapsoides* is therefore believed to be more accurately reflected by the results of the examination of the crabs from Jaro.

A difference in infection rate and metacercarial load may be expected in crabs from the same stream according to size (or age), since a crab that has stayed longer in a stream would have had more chances of exposure to *Paragonimus* cercariæ. In the small sample studied for this purpose (Table 2), the metacercarial load appeared to be heavier among the larger (older) crabs than among the smaller (younger) ones. A similar observation was made by Takahashi,(4) who reported that *E. japonicus* measuring 71 to 80 mm had a higher infection rate and more metacercariæ than the smaller crabs. Yokogawa,(5) however, found no correlation between the size of *E. japonicus* and the infection rate and and he believes that this was because the heavily infected crabs died sooner than the lightly infected ones.

ON INFECTION IN THE HUMAN POPULATION

Eating habits relative to paragonimiasis.—Since the observations in the crabs from Masaili Stream and Agong-ongan River in Barrio Pitogo revealed a more than adequate infective potential to the definitive host and since these crabs are a regular item in the diet of the barrio folk, the prevalence of paragonimiasis among these people will primarily be determined by how these crabs are prepared for eating.

One of the most important findings made in this survey was the discovery that these crabs are eaten by a considerable

segment of these people in the frankly raw state as "kinilao." This eating practice is not confined to Barrio Pitogo, since a number of people from the "poblacion" and other barrios also admitted eating the same dish. There is no doubt whatever that, by the manner that the "kinilao" of crabs is prepared and eaten by these people, whatever *Paragonimus* metacercariæ may be present in these crabs will still be in a viable state when ingested.

The preparation of food as "kinilao" is not peculiar to this locality or to the province of Leyte; many people from other parts of the Philippines prepare a similar dish but the protein base varies from place to place, and vinegar and spices may be used for flavoring instead of "gata" and "calamansi" juice. The protein base of such dishes may be frankly raw or it may be blanched with boiling water. Among the protein foods used for such "kinilao"—type dishes in different areas of the country are pork, beef, fish, squid, etc.

The author has long been aware of the significance of the eating of "kinilao"—type dishes to certain helminthic infections found among Filipinos. For instance, several cases of tæniasis saginata which have been seen at consultation in the Department of Parasitology, Institute of Hygiene, have been traced to the eating of a "kinilao" (or "kilawin" in the dialect of this group of patients) of beef from the carabao, while four cases of intestinal heterophyidiasis were found associated with the eating of another kind of "kinilao"—one prepared from half-cooked fish. The author did not know, however, that river crabs were also prepared as "kinilao" until this study in Leyte.

Another "kinilao" dish—one made from shrimps—is eaten by almost everybody in Barrio Pitogo and other parts of Jaro; however, since no shrimps with *Paragonimus* metacercariæ were found in the stream surveys, this dish most probably does not have anything to do with paragonimiasis in this area.

The eating of roasted crabs "sinugba" presents another possible mechanism for infection with *Paragonimus*. Due to time limitations, it was not possible to find out whether or not *Paragonimus* metacercariæ were still infective after such roasting as done by the people in Barrio Pitogo; however, the admission by some of these people that they removed the crabs from the glowing charcoal before these crabs are apparently thoroughly cooked gives reason to suspect that the crab "sinugba" may play a role, even if a very minor one, in the infection of man with *Paragonimus* in this locality.

Prevalence of paragonimiasis among the inhabitants of Barrio Pitogo.—Two methods of measuring the prevalence of paragonimiasis among the inhabitants of Barrio Pitogo were available: (*1*) by sputum and/or fecal examinations and (*2*) by the intradermal test for paragonimiasis. These two measuring devices gave widely varying results—by the first method, only four out of 83 individuals were found positive, giving a prevalence rate of 4.8 per cent; by the second procedure, a prevalence rate of 33 per cent was arrived at since 31 of 94 people who submitted to this test reacted positively, according to the criteria which were set up for the test. Which of the two rates reflects more accurately the true prevalence of paragonimiasis among the inhabitants of Barrio Pitogo?

The sputum and stool examinations enjoy one distinct advantage over the intradermal test as a measure of prevalence, because positives picked up by sputum and/or fecal examinations are unquestionable, confirmed infections while the intradermal test is subject to the limitations of all such immunologic procedures.

Even under excellent conditions, however, sputum and/or fecal examinations may fail to reveal a high proportion of cases of paragonimiasis because of the inconsistency with which *Paragonimus* ova pass out in the sputum and feces of infected individuals. Notwithstanding highly efficient concentration procedures and using 24-hour sputum collections, Komiya, et al.(1) found that *Paragonimus* ova were demonstrable in only 24 per cent of atypical sputum samples collected from known cases of paragonimiasis while only about one-fourth of these cases consistently showed ova in the feces.

Examination of the sputum and feces, as conducted under field conditions in Pitogo, was performed under the following restrictions:

1. Only one sample was examined from each of the 83 individuals who had a sputum and/or feces examination.
2. Only 12 of these individuals had both a sputum and feces examination. The great majority (62 persons) had only a sputum examination and a small group of 9 persons only had a fecal examination.
3. The sputum specimens examined were not 24-hour collections. Attempts to obtain such samples from 13 individuals produced returns which were definitely not 24-hour collections. For the rest of the 61 sputum examinations, the specimen consisted of a very small amount of sputum that could be hawked up by the subject at the time of the visit to his house.

4. A direct smear and a concentration examination of the sputum were performed for only 13 specimens; for the rest, only a direct smear examination was made.
5. Fecal examination by the acid-ether technic was done for only 21 subjects. Fecal examination in this group of people may lose much of its efficiency as a result of one very common habit especially among adults: the chewing of a concoction of betel nut, a leaf and lime. This induces salivation and an almost incessant spitting. Even the children spit frequently, no doubt in imitation of their elders. This loss of efficiency of the fecal examination was demonstrated in three cases in which the sputum was loaded with eggs of *Paragonimus* while the feces showed a minimal number.

The above considerations lead to the conclusion that many cases of paragonimiasis among the inhabitants of Barrio Pitogo could not have been recognized on the basis of the sputum or feces examination not only because of the inherent limitations of such examinations but also especially because of restrictions imposed by field conditions. It must necessarily follow that the prevalence rate obtained through these examinations must represent only a small fraction of the actual prevalence of paragonimiasis among the inhabitants of Barrio Pitogo.

The intradermal test for paragonimiasis has been in use in Japan for several years. The first report on this test, made by Ritchie, et al.(3) in 1951, was based on the use of a merthiolated extract of the lyophilized adult *Paragonimus westermani* on 87 persons with paragonimiasis and 30 negative controls. Two later reports by Yokogawa, et al.(6, 7) show that the intradermal test has been extensively studied and evaluated since the initial one. Saline extracts of adult *P. westermani* have been utilized in most of these studies, although a veronal-buffered saline extract has also been employed. Other antigens have been tried, including saline extracts of the excretions and secretions of the adult, extracts of the cercariæ and metacercariæ, and even of adult *P. kellicotti.* A number of persons with paragonimiasis and about 6,000 school children in an endemic area have been tested. The antigen has been stored at 4°C for one year and a half without loss of potency. All their experiences with the intradermal test for paragonimiasis have led Yokogawa and his co-workers to the conclusion that the extract of adult *P. westermani,* either in ordinary or veronal-buffered saline, can be reliably employed in screening tests for paragonimiasis in endemic areas.

In the trial of this test among 96 healthy Filipino students, it is significant that there was not one instant where there was

an enlargement of the antigen wheal beyond that of the control wheal in excess of 2 mm; therefore, not one of these individuals who can very safely be considered free of paragonimiasis showed a positive or doubtful reaction to the intradermal test for paragonimiasis. The results obtained here indicate that the criterion of an enlargement of over 4 mm for a positive interpretation is very safe for the dilution used, and reduces to insignificant proportions the occurrence of reactions which may be falsely interpreted as positive among healthy Filipinos.

Some important conditions which the author felt must be explored as possible sources of false positive reactions have also been studied in this work. Of twenty individuals with schistosomiasis japonica who were tested intradermally for paragonimiasis, not one reacted positively to this test although seven of them gave a positive reaction to the skin test for schistosomiasis performed at the same time. It may be tentatively concluded from this small sample of cases that schistosomiasis will not interfere to a significant degree with a screening test for paragonimiasis in areas endemic for both parasitic infections. Table 7 indicates that false positive reactions as a result of tuberculosis will not occur in any significant proportion of individuals tested intradermally for paragonimiasis. Whether or not an active tuberculous process in a patient with paragonimiasis will so depress the reaction to the intradermal test for paragonimiasis as to give a false negative reaction is a question that has yet to be studied.

No attempt was made for a specific study of the relationship, if any, of the common intestinal nematodes (*Ascaris*, hookworm, *Trichuris*) to the intradermal test for paragonimiasis. These parasites are so common in the Philippines that it can be safely assumed that the great majority of the individuals in the schistosomiasis and tuberculosis groups, as well as the inhabitants of Pitogo, have one or more of the three species of worms. Even the student group can be assumed to have about a 50 per cent prevalence rate with one or more of these worms. If infection with any of these common nematodes, therefore, were an important source of false positive reaction to the intradermal test for paragonimiasis, a good proportion, if not the great majority, of the subjects tested in each group would have reacted positively.

The intradermal test, as used among the Japanese, has been shown to have an efficiency close to a hundred per cent. Yoko-

gawa et al.,(7) using the same dilution of the antigen and criteria of interpretation as in this study, found that all of 17 patients with paragonimiasis showed a positive reaction. In the report of Ritchie et al., 95 per cent of 87 persons with paragonimiasis in Japan responded positively to the test, although in this trial an enlargement of 3 mm or more of the antigen wheal beyond that of the control wheal was interpreted as a positive reaction. On that basis, a positive reaction would have been shown by all twelve of the Filipinos with paragonimiasis tested in this work (Table 5). Actually, under the conditions set for the test, only 9 (75 per cent) could be said to have given a positive reaction.

It is realized that the very limited sample of definite cases of paragonimiasis studied here is not sufficient for an accurate evaluation of the efficiency of this test. A larger series must be studied. The results in this sample, however, seem to indicate that the test, as used and interpreted in this survey, may fail to pick up a significant proportion of positives, and that the one serious limitation to the test among Filipinos may be the lack of efficiency in picking up some infected individuals rather than of giving a positive result in an individual who really is not infected with *Paragonimus*.

Considering the finding of the Japanese investigators that the intradermal test is a reliable tool in screening tests for paragonimiasis and our own observations on the absence or great infrequency of false positive reactions among healthy Filipinos or among those with the common intestinal parasitic infections, schistosomiasis or tuberculosis, it becomes extremely difficult not to ascribe the 33 per cent positive reactions among the inhabitants of Barrio Pitogo as being due to infection with *Paragonimus*. The finding that the percentage of positive reactors was significantly higher among those with a history of hemoptysis and among those who admitted eating crab "kinilao" compared with those who denied a history of hemoptysis or eating the raw crab dish gives additional support to the reliability of the intradermal test for paragonimiasis.

Taking the total picture derived from this study in Barrio Pitogo, including the high infective potential present in the crabs, the not uncommon habit among the natives of eating crab "kinilao," the limitations in efficiency of the sputum and feces examinations as a result of field conditions, and the results of the intradermal test for paragonimiasis, the evidence becomes

overwhelmingly in favor of the conclusion that the actual prevalence of paragonimiasis among the inhabitants of that barrio is in the order of magnitude indicated by the intradermal test. It is even possible that the actual amount of infection in this barrio is higher than that indicated by the results of the intradermal test since the test, in our hands, did not give a 100 per cent efficiency in the small sample of twelve definite cases of paragonimiasis tested.

Infected individuals as source of snail infection.—Paragonimus eggs from infected individuals may reach the streams through the sputum and feces. The fecal source, however, does not appear important since the people in Barrio Pitogo do not defecate in the streams. They go to the open fields quite a distance from the water. While certain possibilities may be thought of, such as that the feces may be washed into the streams by rain (which is heavy and frequent), these possibilities appear rather improbable. The sputum source seems to be much more important. Spitting among these people seems to be incessant, a habit abetted by another—the chewing of a betel nut concoction. A native may go to the stream at least once a day—to bathe or wash, fetch water, wash clothes, catch crabs or collect snails or shrimps for food, etc.—and spit as frequently while he is in the stream as when out. It would thus seem that even if only a few infected individuals would be passing out eggs in the sputum at one time, these persons could deposit enough eggs from their sputum in the streams as to keep up the infection in the snail.

INFECTION IN DOMESTIC CATS

The finding that four out of five domestic cats from Barrio Pitogo were infected with *Paragonimus* indicates that, as in Casiguran, a high proportion of these cats are definitive hosts of the fluke in the endemic area.

The possibility has already been advanced in the report of the Casiguran survey that, in that endemic locality, paragonimiasis may be primarily an enzootic, with man coming into the biological cycle of the parasite only probably by accidental means; however, the possibility was also mentioned that the domestic cat may only be a blind alley in the life cycle of *Paragonimus* because its defecation habits do not usually bring it near the water.

The prevalence of paragonimiasis among the inhabitants of Barrio Pitogo as well as the habits of these people clearly

point to the maintenance of *Paragonimus* in this locality through a man-snail-crab-man cycle. The finding that a high proportion of domestic cats in this area may also receive the parasite from the crab opens the way to the thought that another (and interrelated) cycle, a cat-snail-crab-cat one, may be going on simultaneously. While *Paragonimus* ova are passed out in the feces of infected cats, there is no evidence to show that these ova reach the streams in sufficient numbers as to result in infection of the snail host; therefore, the importance of the domestic cat as a reservoir host of the parasite must remain unresolved at this time. Apparently, however, the life cycle of *Paragonimus* could easily be maintain in Barrio Pitogo even if man were the only definitive host available.

SUMMARY AND CONCLUSIONS

(1) On the basis of both published and unpublished reports that cases of paragonimiasis have been found among the people of the Municipality of Jaro, Leyte Province, a survey to gather epidemiologic data on paragonimiasis in this area was made in October, 1955. Jaro is similar to Casiguran, Sorsogon, in having a hilly topography and a climate characterized by a heavy rainfall and no distinct dry season. Interest became centered on one of the barrios of Jaro (Barrio Pitogo) where the socio-economic conditions of the inhabitants were very similar to those obtaining in the three barrios of Casiguran which had been studied previously.

(2) Fresh-water crabs apparently identical with *P.* (*B.*) *grapsoides* were found in all sixteen streams and rivers which were surveyed in Jaro and vicinity. In twelve of these waters, *P.* (*B.*) *grapsoides* with *Paragonimus* metacercariæ were found. The snail, *B. asperata,* was also present in six of the streams, as determined merely by cursory search.

(3) Detailed studies on infection in *P.* (*B.*) *grapsoides* from Masaili Stream and Agong-ongan River in Barrio Pitogo were carried out. The results show that any definitive host in the habit of eating these crabs in the raw or inadequately cooked condition will most likely be infected with *Paragonimus.*

a. 64 (98.5 per cent) of 65 crabs from Masaili Stream and 24 (47.2 per cent) of 51 from Agong-ongan River were found positive for *Paragonimus* metacercariæ. The metacercarial load in 36 positive crabs from Masaili Stream varied from 1 to 112, with a median of 12 and a mean of 27. The metacercarial load of the crabs from Agong-ongan River was apparently lighter.

b. The localization of *Paragonimus* metacercariæ in *P.* (*B.*) *grapsoides* from Masaili Stream can be expressed as: body muscles> heart and pericardium> gills. Such localization is significantly different from that in *E. japonicus* (the important crab host in Japan), in which the localization is: gills> body muscles> heart and pericardium, with almost no metacercariæ in the last named situation.

c. Larger (older) *P.* (*B.*) *grapsoides* apparently had a heavier metacercarial load than the smaller (younger) ones.

(4) Crabs obtained from the two streams in Barrio Pitogo are a common and important dietary item among these people. Practically all of them eat these crabs after boiling. A significant proportion (40 per cent) of 97 residents interviewed in this study, however, also admitted eating the crab in the frankly raw state as "kinilao." In addition, crabs are also generally eaten as "sinugba," the crabs being roasted in a manner which may possibly leave some viable metacercariæ.

(5) Sputum and/or fecal examination of 83 residents of Barrio Pitogo showed only 4 (4.8 per cent) positive for *Paragonimus* ova; however, 31 (33 per cent) of 94 persons, which included the 83 with sputum and/or fecal examination, showed a positive reaction to the intradermal test for paragonimiasis. The percentage of positive reactors was significantly higher among those with a history of hemoptysis and among those who admitted eating crab "kinilao" than among those who denied a history of hemoptysis or eating crab "kinilao."

(6) Because of restrictions imposed by field conditions, especially the fact that only a direct smear examination of the very small amount of sputum sample hawked up by a subject at the time of visit to his house could be performed for the great majority of subjects, much of the efficiency of the microscopic examination for *Paragonimus* ova was lost; consequently, such examination could have revealed only a small fraction of actual infection present.

(7) Studies on the intradermal test for paragonimiasis among a limited number of Filipinos indicated that:

a. False positive reactions will not occur or will be rare among healthy individuals (free from paragonimiasis).

b. The intradermal test, as used and interpreted, may fail to pick up about one-fourth of *Paragonimus* infections.

c. False positive reactions to the test as a result of schistosomiasis japonica or tuberculosis will not occur to any significant degree; therefore, these two condtions will not interfere with the use of the intradermal test in screening surveys in the Philippines.

d. The common intestinal nematodes (*Ascaris*, hookworm, *Trichuris*) are not important sources of false positive reactions to the test.

(8) From considering all the information obtained in this study, including the high percentage of infection and the metacercarial load in crabs, the crab-eating habits of the people, the impairment in efficiency of the sputum and feces examinations as performed in the field, and the results and apparent reliability of the intradermal test for paragonimiasis, it is concluded that the actual prevalence of paragonimiasis in the population of Barrio Pitogo is in the order of magnitude indicated by the intradermal test or higher.

(9) Infection with *Paragonimus* is apparently very common in domestic cats in Barrio Pitogo. The role of the cat as a reservoir host of the parasite cannot yet be assessed because of lack of information on the transfer of the parasite from the cat to the snail. In Barrio Pitogo, spitting is a very common habit; among the adults, this is at least partly due to the chewing of betel nut. A complete man-snail-crab-man cycle thus exists for the maintenance of the parasite in this locality.

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ILLUSTRATIONS

PLATE 1

(Views of Barrio Pitogo, Jaro)

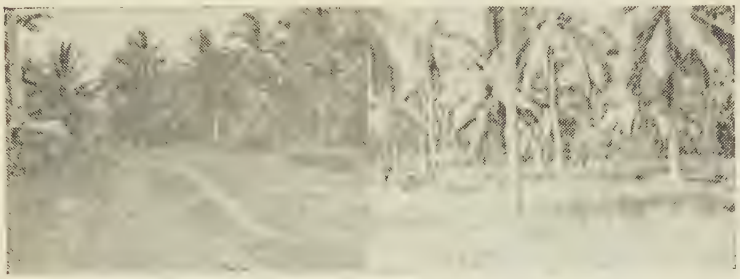
FIG. 1. The trail leading to the barrio center.
2. A typical portion of the barrio.
3. The center of the barrio.
4. The "lo-on," where coconut is smoked and dried to make copra.
5. Portion of Cabiyongan River.
6. Portion of Masaili Stream, Barrio Pitogo.
7. *Brotia asperata* on top of big stone in Masaili Stream.

PLATE 2

FIG. 1. Positive reaction (non-amoeboid) to the intradermal test for paragonimiasis.
2. Positive (amoeboid) reaction to the intradermal test for paragonimiasis.
3. Another subject showing a positive amoeboid reaction to the intradermal test for paragonimiasis.
4. The intradermal tests for paragonimiasis and schistosomiasis in a subject with schistosomiasis japonica (Subject no. 7 of Table 6.)

TEXT FIGURE

The location of the sixteen streams and rivers surveyed in Jaro and vicinity. In the streams indicated by a circle, *P.* (*B.*) *grapsoides* harboring *Paragonimus* metacercariæ were present; no infected *P.* (*B.*) *grapsoides* were found in the streams indicated by a triangle.



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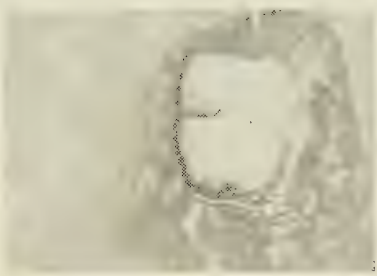

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PLATE 1.

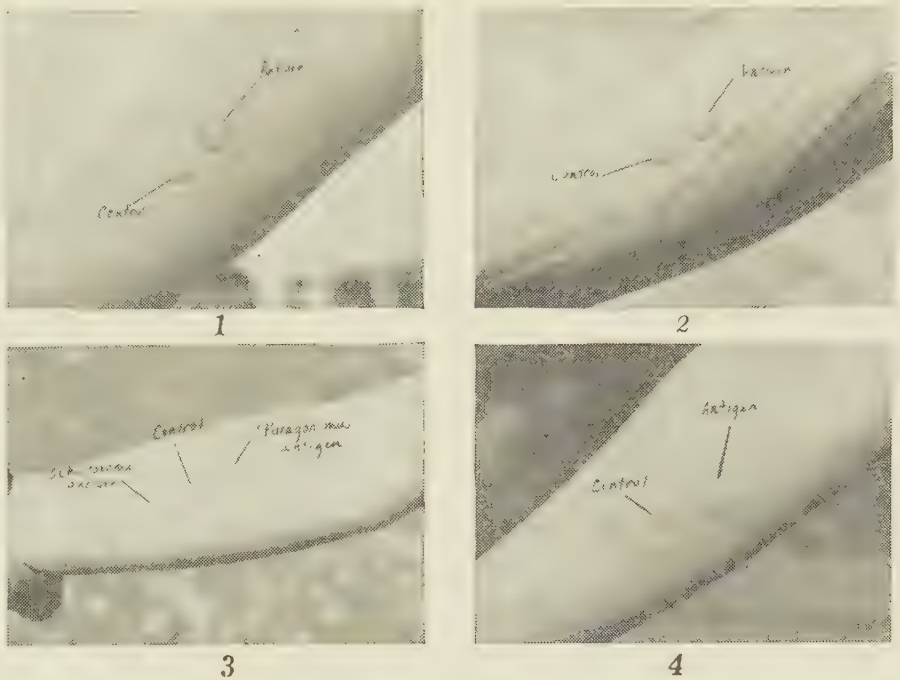

PLATE 2.

SEASONAL CHANGES IN THE GONADS OF HILSA ILISHA HAMILTON

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TWO PLATES AND ONE TEXT FIGURE

INTRODUCTION

The Indian shad, *Hilsa ilisha* (Ham.), which is one of the most valuable food fishes of India, has received considerable attention from Indian zoologists as well as fishery biologists since 1878. Since then much attention has been focussed on the study of the breeding and bionomics of this fish by several authors. Determination of the spawning ground and breeding season of *Hilsa ilisha* (Ham.) were made by Day (1873), Hora (1938), Jenkins (1938), Hora and Nair (1940), Chacko and Ganapati (1949) and Jones and Menon (1950). These efforts have, no doubt, improved our knowledge of the subject.

A series of catches of the post-larval stages of *Hilsa* by Nair (1939) led Hora and Nair (1940) to conclude that this fish bred in the Hooghly River all the year round and with a greater frequency in the rainy season. But, Jones and Menon (1950) could not corroborate this finding because in their routine plankton hauls in the Hooghly during the winter months, the pro-larvæ and post-larvæ of *Hilsa* were absent. These authors (1951) state from this observation that "the breeding of *Hilsa ilisha* (Ham.) in Hooghly River is very restricted, if not at a standstill, during the winter months of December and January . . . However, it is quite possible that the fish may even then be breeding lower down in the estuary, where the temperature is higher." Investigations also show that this fish breeds in the Chilka Lake mainly during the monsoon months (July to October) when the Daya River and its tributaries are in flood.

A detailed study of the existing literature on the breeding of *Hilsa ilisha* (Ham.) will show that the spawning period of *Hilsa* has been inferred mainly from collections made of eggs

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or young ones, but direct observations on the spawning time of this species could not be made because it does not breed in stagnant waters. Moreover, as *Hilsa* occurs in widely separated zones in India and lives under diverse ecological conditions, there is a possibility, as suggested by Jenkins (1940) and supported by Job (1942) that "it comprises several 'races' capable of living and breeding under different environmental conditions.' Job is of the opinion that it is likely that the breeding period of *Hilsa* is slightly variable from place to place on account of different ecological conditions and that an exact knowledge of its breeding in a particular territory is, therefore, necessary in order to enable the fishery department to frame and apply the necessary restrictive laws in that territory.

No attempt has previously been made to determine the exact breeding period of *Hilsa ilisha* (Ham.) at any particular place by following the gross and microscopic changes in the gonads of this fish all the year round. In this paper, the author has attempted to determine the exact breeding time of this fish in the river Ganges at Banaras by studying its annual reproductive cycle. The gross and microscopic changes observed in the testes and the ovary of *Hilsa ilisha* (Ham.) during the twelve months of the year (from November, 1952 to October, 1953) at Banaras are given here. From these the exact knowledge of its breeding season in this state can be deduced.

The reproductive cycles of fishes have been studied by several investigators, the more important among the species investigated being the following:

Author	Species investigated
Turner (1919)	*Perca flavescens.* Male
Geiser (1922)	*Gambusia affinis.* Male
Foley (1926)	*Umbra limi.* Male
Hann (1927)	*Cottus biardii.* Male and female
Kulaev (1927)	*Perca fluviatilis.* Male
Mien (1927)	*Perca fluviatilis.* Female
Craig-Bennet (1931)	*Gasterosteus aculeatus.* Male and female
Hickling (1935)	*Merlucius merlucius* L. female
Bennington (1936)	*Betta splendens.* Male
Wiart (1936)	Teleosteans in general. Male
Mathews (1938)	*Fundulus heteroclitus.* Male and female
Bullough (1939)	*Phoxinus lævis.* Male and female
Suzuki (1939)	*Plecoglossus altiveles.* Female
Guerbilsky (1939)	*Mirror carp.* Female

Mendoza (1939)	*Neotoca bilineata.* Female
Mendoza (1940)	*Neotoca bilineata.* Female
Mendoza (1941)	*Neotoca bilineata.* Female
Frederick (1941)	*Galeichthys felis.* Male
Jones (1940)	*Salmo salar.* Male
Mendoza (1943)	*Neotoca bilineata.* Female
Weisel (1943)	*Oncorhynchus nerka.* Male
Jost (1943)	*Callionymus lyra.* Male
Hickling (1945)	*Sardina pilchardus* Walbaum. Male and female.
James (1946)	*Lepomis macrochirus* and *Huro salmoides.* Male and female
Ghosh & Kar (1952)	*Heteropneustes fossilis.* Male and female
Zaitzev (1950)	Pike. Male

A very exhaustive and comprehensive recent review of the literature on the seasonal changes in the gonads of fishes is given in the work of Ghosh and Kar (1952). These authors, working on *Heteropneustes fossilis,* failed to notice any seasonal changes in the testes but found a marked cyclical activity in the ovaries.

MATERIAL AND METHODS

The material for the present study was procured from the river Ganges at Banaras. Collections of live specimens of *Hilsa ilisha* (Ham.), both males and females, were made from the beginning of October, 1951 to the end of October, 1953. The normal seasonal cycle was studied by examining the condition of the gonads of a large number of specimens secured in the different months of the year throughout this period. As a rule only full grown specimens measuring on the average 33 to 37 centimeters in length were used in this work. Five to ten specimens were usually examined every week and dissections of the fish were made at the river bank to avoid post-mortem changes.

The fishes were generally killed by decerebration after which the gonads were dissected out carefully. Pieces of the gonads were fixed immediately in various fixatives like Bouin's fluid and Zenker, for studying the histological details. Paraffin sections were cut 6 to 8 micra thick and stained with iron haematoxylin and eosin. Mallory's triple stain with Anderson's mordant was also used to study the connective tissue in the gonads. Simultaneously, the gonads of some of the specimens were dissected out completely, their volume determined and preserved in 4 per cent formol for sketching.

STRUCTURE OF THE GONADS

Testes.—The testes of *Hilsa ilisha* (Ham.) are elongate, dorsoventrally compressed bodies, whitish in color in the breeding season, but translucent or flesh-colored in the off-season. They lie in the body cavity below the kidneys suspended in the mesorchium. They are roughly elliptical in cross-section but more flattened on their dorsal side. The spermatic duct run close to the inner borders of the testes and emerge out as a pair of narrow ducts which run for some distance caudally and finally unite before opening to the exterior through the male genital opening in the cloaca. The urinary ducts open separately into the cloaca.

The testes are covered with theca and are made up of a number of seminiferous tubules. Each tubule contains a number of groups of germ cells, or "clusters," the germ cells in each 'cluster' being in the same stage of maturity. As the testes enlarge on the advent of the breeding season, the seminiferous tubules also grow in diameter and the adjacent tubules show a tendency to communicate with one another on account of the fusion of their contiguous walls at certain points resulting in a '*syntubular*' condition.

Ovary.—The female reproductive organs of *Hilsa ilisha* (Ham.) consist of a pair of ovaries occupying the same position as the testes in relation to other organs in the body cavity but suspended from the dorsal side by the mesovarium. The ovaries of *Hilsa* measure approximately 15.5 centimeters in length and 2.7 centimeters in width at the broadest point in the breeding season and 10.2 centimeters by 0.5 centimeter in the off-season. They are more or less cylindrical in shape with the ovarian vein closely applied to the dorsal side. The ovaries are translucent and slightly pinkish in color in the off-season, but yellowish in tinge in the breeding season. The oviducts are continuations of the basal portions of the ovaries and are very short. They join together shortly before opening into the cloaca through the female genital opening. The urinary ducts open separately into the cloaca.

Within the outer wall of the ovary are many ovigerous lamellæ, that are, in fact, folds of the ovarian wall, bearing the germinal epithelium on their inner side.

GROSS SEASONAL CHANGES IN THE GONADS

From an examination of the gonads of *Hilsa ilisha* (Ham.) collected at various times in the year, it is clear that the

volume of the testes and the ovary changes cyclically. The changes in the volume of the gonads in the different months of the year are recorded in Table 1. These changes, especially in the ovary, serve as an index of the spawning activity of the

TABLE 1.—*Measurements of the average volume of the testes and the ovaries and the diameter of the ova of Hilsa ilisha (Ham.) in the different months of the year. The last two columns show the mean monthly water temperature of the River Ganges at Banaras and the average monthly rainfall in the locality during the year 1952–1953.*

Month and year	Average volume of testes	Average volume of ovary	Average diameter of ova	Average monthly water temperature	Average monthly rainfall *	
	cc	*cc*	*mm*	*°C*	*inches*	*mm*
December, 1952	1.50	5.00	0.054	19.0	0	0
January, 1953	2.25	12.50	0.099	19.6	1.05	26.67
February, 1953	3.25	19.00	0.142	20.5	0.34	8.636
March, 1953	3.50	39.00	0.213	25.0	0	0
April, 1953	1.25	13.00	0.078	27.3	0	0
May, 1953	0.50	6.00	0.056	30.0	0	0
June, 1953	0.50	7.00	0.159	29.4	3.52	89.408
July, 1953	6.66	96.50	0.275	28.8	12.27	311.658
August, 1953	7.00	125.00	0.311	28.7	18.56	471.424
September, 1953	5.00	70.50	0.200	28.2	12.99	329.946
October, 1953	4.075	4.00	0.086	27.4	0.84	21.336
November, 1952	1.00	3.00	0.080	19.1	0	0

* Obtained from the Indian Meteorological Station, Poona.

fish as suggested by Hickling and Rutenberg (1936). The data in Table 1, when plotted, yield a bimodal curve (Fig. 1) showing that both gonads enlarge twice in the year. The measurements of the ova given in the third column of Table 1 also confirm this result. To make sure that the measurements of the ova were taken at the maximum diameter, only those sectioned eggs, in which the nucleus was visible, were measured.

SEASONAL HISTOLOGICAL CHANGES IN THE GONADS

The principal histological changes occurring in the gonads of *Hilsa ilisha* (Ham.) at different times in the year are mentioned below to sustain the results obtained from the study of the macroscopic changes affecting the gonads.

TESTIS

December.—The testis tubules are full of mature resting spermatogonial clusters (Plate 1, fig. 1), though the total volume of the testis does not show an increase from that of the preceding month. Mature spermatogonia are spherical in shape, possess a large round nucleus and a prominent darkly-staining nu-

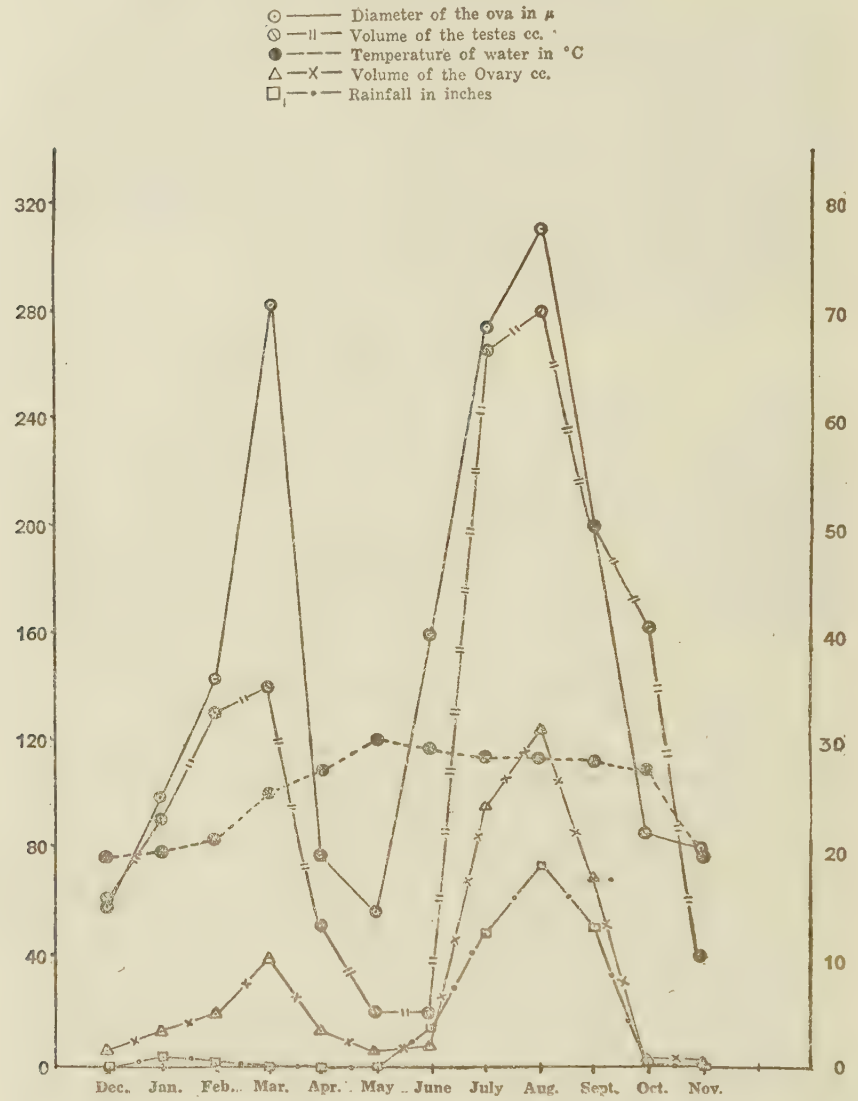

Fig. 1. Seasonal variations in the volume of the testes and ovary, the diameter of the ova of *Hilsa ilisha* (Ham.), the water temperature of Ganges and rainfall of Banaras during the different months from November 1952 to October, 1953.

cleolus. Small, round or slightly oval, interstitial cells with a darkly-staining nucleolus are noticeable in the intertubular spaces.

January.—In the early part of this month, some of the clusters of germ cells lying in the center of the tubule, which are

in a more advanced state of spermatogenesis, dissolve (Plate 1, fig. 2) and produce a lumen. The whole of the testis is, however, not in the same phase of spermatogenetic activity at the same time, the portion adjacent to the spermatic duct being more advanced than the rest of it. Generally each cluster contains germ cells in nearly the same stage of development. As each cluster attains the final stage of spermatogenesis, the thin limiting membrane bounding it gives way, thus liberating the sperms into the lumen and also enlarging it a step further. The intertubular spaces contain the blood vessels, elastic fibers and a few interstitial cells. The number of the latter has now become much reduced compared with that in December.

February and March.—The histological changes taking place during these months are: (*1*) an increase in the number of spermatocytes, spermatids and sperms, (*2*) increase in the diameter of the seminiferous tubules and consequent narrowing of the intertubular connective tissue and spaces, and (*3*) the disorganization of most of the clusters (Plate 1, fig. 3). The histology of the testis in March reveals peak spermatogenetic activity. The tubular lumen in some cases is also partially empty (Plate 1, fig. 4), obviously owing to the discharge of the spermatozoa to the outside. Interstitial cells become unnoticeable now.

April.—In this month, there is a slowing down of spermatogenesis and the seminiferous tubules expel most of its spermatozoal content by the pronounced hypertrophy of the intertubular connective tissue. Consequently, there is a shortening of the tubular lumen. However, a small amount of residuary sperms may remain in the tubular lumen (Plate 1, fig. 5). The interstitial cells show a relative increase in number and become noticeable once again.

May.—The tubules regain their proper shape, (Plate 1, fig. 6) the clusters of spermatogonial cells reappear in the tubules. Henceforth, the clusters, by the multiplication of the spermatogonia, enlarge as a result of which the tubules increase in diameter. Narrowing of the intertubular connective tissue occurs due to the collateral pressure exerted by the enlarging seminiferous tubules.

June.—There is a marked increase in spermatogenetic activity at this time. Some of the adjacent tubules fuse with one another and their lumen consequently become confluent.

The intertubular connective tissue is thinned out and spermatocytes and spermatids abound in the seminiferous tubules (Plate 1, fig. 7). By the end of June, large quantity of sperms is seen in the tubular lumen. Interstitial cells once again disappear from the intertubular spaces. Resting spermatogonia either singly or in clusters linger on the wall of the tubules.

July, August, and September.—In this part of the year there is a preponderance of sperms in the seminiferous tubules whose lumen are wide and, in many cases, confluent (Plate 1, fig. 8). As the tubules become partially empty, the resting clusters divide quickly to produce more sperms. The sperm production seems to be at its peak again in August. The interstitial cells are less evident owing to the extreme narrowing of the intertubular spaces.

October.—During the first half of this month expulsion of residuary sperms takes place and the intertubular connective tissue is hypertrophied to a great extent. As suggested by Turner (1919), the hypertrophy of this tissue perhaps facilitates the expulsion of sperms.

November.—The testis in the beginning of this month is wholly 'spent'. Some of the cells lining the tubular wall show signs of necrosis and desquamation and dissolution of part of the contents of the tubule takes place (Plate 1, figs. 9 and 10). On closer scrutiny of the tubular contents, however, one can find certain isolated but healthy mother cells that are, more or less, round and large and possessed of a clear nucleoplasm, and a centrally situated darkly-staining nucleolus. Obviously, these are the reserve germ cells mentioned by Hann (1927) in *Cottus biardii* (cf. bibliog. p. 2) which are destined to give rise to the spermatogonia for the next cycle.

The hypertrophy of the intertubular connective tissue at this stage is only a temporary feature. By the end of November, it is seen that this tissue recedes considerably and the seminiferous tubules become reconstituted in preparation for the next cycle of spermatogenesis. Wiart's (1936) belief that the hypertrophied interfollicular tissue sends off a web of fibers to the interior of the lumen of the tubules exerting a kind of chemotropism on the nondischarged spermatozoa which are drawn towards the vacuoles formed by the web of the interfollicular connective tissue, where they suffer phagocytosis, is not supported by any evidence. No such thing was observed in the present case.

THE OVARY[1]

November and December.—In these two wintry months of the year the ovary is in the resting state as shown by its histological condition. The ovary is full of young oocytes mostly in Stage I of James (1946) (Plate 2, fig. 9). The average diameter of the oocytes is 72 micra. The characteristics of the majority of oocytes at this stage are: (*1*) size is small, (*2*) shape is irregular, (*3*) nucleus is round or slightly oval, and (*4*) the nucleoli are distributed at the periphery of the nucleus (germinal vesicle). Towards the end of December, some of the oocytes increase in diameter, their germinal vesicle becomes larger, and an increase in the number of the nucleoli is evident. A layer of thin flat cells with slightly oval nuclei surrounding the oocyte constitutes the follicular epithelium.

January.—The oocytes in this month show greater differentiation by the appearance of yolk vesicles (Plate 2, fig. 1). The average diameter of the oocytes at this stage is 99 micra. This corresponds to Stage II of James (1946). By the end of January one also finds the formation of a darkly staining yolk-nucleuslike body ejected into the cytoplasm from inside the germinal vesicle, the appearance of which being an indication of the approaching yolk formation. In general appearance the yolk-nucleus resembles the darkly staining nucleolus of the germinal vesicle. As the yolk nucleus travels to the surface of the cell, it breaks up and vanishes there and then the yolk makes its appearance in the peripheral vesicles (vacuoles of the oocytes cytoplasm).

February.—In this month most of the oocytes are found in Stage III of James,(34) for the yolk has appeared in the vesicles of majority of them (Plate 2, fig. 2). The follicular epithelium remains unchanged. The theca layers are also not clearly visible in this fish. Towards the end of February, the germinal vesicles of many of these oocytes assume an irregular outline, and the oocyte measures, on the average, 142 micra in diameter.

March.—Majority of oocytes in this month attain an average diameter of 213 micra, the maximum size reached in its first cycle. The oocytes now can be said to be in Stage IV of James,

[1] For the sake of convenience in description, the nomenclature used by James (1946) for designating the different stages in the maturation of oocytes is used here to denote the histological changes occurring in the ovary.

though a few may also be found in Stages I, II, and III. The whole cytoplasm of the cell is full of large-size yolk globules. The follicular epithelium is so thin that it is difficult to distinguish the cells constituting it. Besides the yolk globules, empty vesicles are also seen in the cytoplasm (Plate 2, fig. 3). They probably contained fat or oil globules, which might have dissolved in the course of processing. The germinal vesicle lies somewhat eccentrically in many of the ova.

April and May.—The ovary contracts in volume in these months (Plate 2, fig. 4) owing to three causes as evident from their histology: (*a*) Most of the ova which were in Stage IV in the preceding month are found to be in a state of resorption, Stage V of James; (*b*) A temporary stoppage of the growth and maturity of oocytes of Stages I and II; and (*c*) Collapse of a few follicles due to the discharge of the contents. The last mentioned cause, however, contributes only little towards the reduction of the volume of the ovary, for empty follicles are found only here and there. Sometimes it may be that another set of small oocytes may attain Stage IV in development, but turn atretic instead of spawning. The average diameter of oocytes in May is only from 56 to 62 micra.

June.—Towards the middle of this month, many of the oocytes are found again in Stage III, distinguished by the presence of yolk in the vesicles. This condition resembles that in February.

July, August, and September.—The ovary in these months is practically full of mature ova (Stage IV). (Plate 2, fig. 6). However, a few oocytes in Stages II and III may also be present. These mature quickly and replace the ova that have escaped out. During these months, Stage IV oocytes (mature ova) measure, on an average, 302 micra in diameter. It is worth mentioning here that the diameter of the Stage IV oocytes now is greater than that attained in March. (Table 1 and fig. 1)

October.—The ovary in this month suffers from recession to a marked extent. The histological feature of the ovary at this time is its "spent condition" (Plate 2, fig. 7). Its prominent feature is the presence of a large number of empty follicles showing the so-called *corpora lutea* (Plate 2, fig. 8). A few ova, nevertheless, are found in a state of resorption. The difference between an empty follicle showing signs of the so-called *corpus luteum* and an ovum undergoing resorption is very marked as

may be seen by comparing Plate 2, figs. 4 and 8. In the former there is a definite space left behind by the ovum which has escaped out, though this tends to become reduced by the hypertrophy of the follicular wall, whereas in the latter such space does not occur.

Towards the end of October, the ovary again becomes filled with small oocytes most of which, however, remain dormant until the next January, when the cycle is repeated.

DISCUSSION

From the foregoing descriptions, it is clear that the gonads of *Hilsa ilisha* (Ham.) hypertrophy twice in the year, resulting in the enlargement of their volume once in February and March and again in July and August. A study of Fig. 1 also reveals that the minimum volume of the gonads is reached first in May and again in the period November to December. The histological study of the gonads throughout the year has shown that the testes enlarge and enter upon active spermatogenesis by February lasting until March. Correspondingly, the ovary also progressively enlarges maximally by February and March after which both the testes and the ovary decline in volume. The bulk of the sperms having been discharged, the testis enters upon a resting phase by May. This constitutes the first cycle ending in May.

The bimodal curve of the ovary and the testes is likely to make one think that spawning occurs twice during the year. But a close histological study of the gonads during the twelve months of the year has revealed that the spawning of ova does not take place in the first cycle in February and March. Instead of spawning and depositing the ova, the mature ova of the first cycle turn atretic within their follicles. This means that there is hardly any crop of "brood" in the first reproductive cycle. After atresia and the absorption of its by-products, the ovary contracts in volume and with the residuary ova pass into the "quiescent phase."

The next peak of oogenetic and spermatogenetic activity occurs in August, but the crest of the wave extends over a period of three months, *i.e.*, from July to September. The gonadal volume undergoes reduction a second time in November and December. In October the volume of the ovary becomes reduced on account of the expulsion of most of the mature ova and the cessation of the maturation process of fresh oocytes.

At this time the ovary shows a number of collapsed follicles from which the ova have escaped, although the follicular wall shows hypertrophy forming the *corpus luteum*. However, a few residuary ova undergoing resorption may also occur. There is a sharp difference between the two types of follicles. The follicles of the ovary at this time (October) clearly indicate that ovulation has taken place in the preceding months. The absence of atretic ova at this time of the year lends further support to the above conclusion. This is markedly different from what happens in February and March, when no spawning takes place and the majority of the ova degenerate by atresia. In the second cycle, extrusion of ova takes place, and successive crops of ova are produced until the end of the season when contraction of the ovary and cessation of its productive capacity become imminent. Though the spawning continues from July onward in the second cycle, the empty follicles are scarcely seen until the close of the spawning period, on account of the maturation of the successive "broods."

Clark (1934) attempted to study the maturity of the California sardine (*Sardinia coerulea*) by measuring the diameter of the ova and noting its frequency. Later Hickling and Rutenberg (1936) fixed the spawning periods in hake, haddock, pilchard, herring and Lepidogaster by the same method. More recently, De Jong (1939) has, in a similar way, investigated the spawning habits of 13 species of teleostean fishes from Java Sea. In India also recently this method has been employed by a few workers to find out the spawning period of fishes, namely, Karandikar and Palekar (1950) in *Polynemus tetradactylus*, Palekar and Karandikar (1952) in *Thrisocles purava* and Prabhu (1956) in nine species of fishes.

A study of the reproductive cycle of *Hilsa* has revealed that the above method is not a sure and reliable one for determining the exact spawning period of a fish in a particular place. Although the ovaries may macroscopically show an enlarged condition and microscopically show the majority of the oocytes in Stage IV, still it is possible that the majority of them, if not all, may turn atretic subsequently as has been found in the case of the March "brood" of *Hilsa*.

On the other hand, the presence of a large number of collapsed follicles from which the ova have escaped, will be a positive proof of the spawning that had taken place just before, if it is supported by the peak activity of the gonads at that time.

The absence or scanty presence of atretic follicles after the ovaries reach a peak activity will be an additional proof that spawning has taken place. Therefore, the present author's observations corroborate the view of Cunningham (1897) who sums up the structure of the "spent" ovary thus: "The essential characteristic of 'spent' ovaries is, therefore, the presence of empty collapsed follicles from which the eggs have escaped."

A study of the temperature of the water of the Ganges at Banaras (Table 1 and Fig. 1) and its correlation with the principal histological changes in the gonads throughout the year has shown that the temperature probably exerts some influence upon the ripening of the gonads. Again, a study of the last column of Table 1 and an examination of Fig. 1, showing the monthly rainfall at Banaras for the year 1952–1953 indicates that the rainfall is nil in March and sufficient in August and September (monsoon months in Banaras) to provide favorable conditions for spawning.

The extant literature on the breeding habits of *Hilsa* shows that opinion is divided on this point and in a certain degree conflict exists in the opinions expressed by the workers on this subject. For example, Hora (1938), Hora and Nair (1940) and Nazir Ahmad (1954) are of the opinion that *Hilsa* breeds throughout the year with a peak in the rainy season and a second peak in February or March in the estuarine waters of Bengal. Chacko and Ganapati (1949) and Jones and Menon (1951), however, think that the breeding is restricted to the rainy season. Hora and Nair (1940) believe that a second reproductive cycle, at the end of the winter, obtains in the case of *Hilsa*. But no convincing argument has yet been produced to define and fix its productive timings. The available evidence mostly consists of the occasional catches of eggs, fingerlings or gravid migrating adults. The month-to-month examination of the gonads of this fish and the histological assessment of their condition were never attempted before. Therefore, the results obtained by the present author have some significance.

It is clear from the findings that the so-called first cycle of reproduction in *Hilsa*, as observed in Banaras, is largely abortive because of the large-scale atresia of the contents of the ovarian follicles. Further evidence of the abortiveness of the first cycle in February and March is also to be had from the study of the diameter of the ova in different months of the

year. It has been found that the maximum diameter of the ova in the second cycle, is greater than that in the first. From the facts of atresia, it is possible to say that in the first cycle the majority of the ova neither attain their maximum growth nor help in reproduction, but suffer destruction. This, however, does not rule out the possibility of occasionally a few ova escaping into the water and becoming fertilized by the water-borne spermatozoa of the male. Thus, if a few young ones become available, they should be treated as the result of an accident. The main reproductive cycle of *Hilsa ilisha* (Ham.) in Banaras therefore, is the second cycle, which coincides approximately with the rainy season. During this cycle the ova do not suffer from large-scale degenerative atresia. This assures a plentiful supply of ripe ova spawned by the mother fish to be fertilized by the milt of the males carried by streams in or near the breeding grounds. It is this second reproductive cycle of *Hilsa ilisha* (Ham.) at Banaras that produces the main crop of fingerlings.

It has been averred by Hora and Nair, Jones and Menon, and Chacko and Ganapati that the principal reproductive cycle of *Hilsa ilisha* (Ham.) is in the rainy season because at this time the rivers are in spate, making migration and selection of spawning ground possible for the fish. The turbidity of water, the rise in temperature and the soft patter of rain provide the conditions that are conducive to breeding and, therefore, the breeding cycle of this fish is so timed as to coincide with the rainy season.

Craig-Bennet (1931), Hoover and Hubbard (1937), Mathews (1939), Burger (1939, 1940), Bullough (1939, 1941), Merriman and Schedl (1941), and Harrington, Jr. (1950) have published experimental data concerning the effects of light and temperature on the sexual cycle of fishes. Mathews (1939) experimenting with *Fundulus heteroclitus* found that "the presence of light is not essential for the complete activation of the testes of *Fundulus* and the low temperature exerts a retarding influence on the maturation of the sperms," implying thereby that warmer conditions of water should prove favorable for reproduction. Burger (1939) experimenting with the same fish corroborates the view of Mathews.

From Table 1, it will be seen that the average temperature is lowest in November, December, January, although there is a slight increase at the end of January. The reproductive activity of *H. ilisha* is almost nil during these months. On

the other hand, when the temperature has risen to 25° C in March, the spermatogenetic and oogenetic activitis are in full swing. Therefore, there may be something in what Mathews says in regard to the role of temperature as an inductive agent causing the onset of reproductive activity. However, it is worth mentioning here that a temperature of 30°C in May (Table 1) perhaps has also an inhibiting effect on the gonadal activity as the histology of the gonad reveals at that time. It is likely, therefore, that in this case both low and high temperatures exert an inhibiting effect on gonadal activity.

SUMMARY

1. The morphological and histological structures of the gonads of *Hilsa ilisha* (Ham.) have been studied.

2. Volume changes in the gonads of this fish followed during the twelve month of the year show that both the testes and ovary of this fish at Banaras attain two peaks, once in March and again in August. The corresponding lowest points in the volume curve are reached first in May and then in December.

3. The histological study of the gonads in the different months of the year reveals that spermatogenesis and oogenesis start by the end of January and reach a peak in March when one finds the ovary full of apparently mature ova and the testes tubules full of mature sperms. After this there is a retardation of the gonadal activity until the end of May or beginning of June. In May gonads are found generally in a resting condition.

4. After the first peak of gonadal activity in March, the testes expel most of its spermatozoa; but in the case of the ovary, the majority of the ova, instead of passing out, undergo atresia and resorption.

5. By June the gonads again begin their activity and this goes to a peak by August. But this time both the ova and the sperms are expelled out resulting in the fertilization of the ova by the sperms in the water. The expulsion of the ova is evidenced by the presence of empty follicles that show signs of the so-called *corpus luteum*. The testes tubules are also found depleted in the months of October and November. After spawning the gonads again pass into a "quiescent phase" and remain in that condition until the following January when the cycles are again repeated.

6. By correlating the variation of the water temperature of Ganges near Banaras with the gonadal activity throughout the

year, it was noticed that very low and very high temperatures perhaps have an inhibitory effect on this activity.

7. The rainy season, which floods the rivers and makes the water turbid and the current flow faster, is thought to be the condition most favorable for the spawning of *Hilsa ilisha* or the Indian shad.

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ILLUSTRATIONS

[Abbreviations: *atr. ovm*, atretic ovum; *bl. ves*, blood vessel; *dis. foll*, discharged follicle (follicle from which the ovum has escaped); *int. cl*, interstitial cell; *int. con*, inter-tubular connective tissue; *ncr. cl*, necrotic cell; *ocy, I*, oocyte Stage I; *ocy. II*, oocyte Stage II; *ocy. III*, oocyte Stage *III; ocy. IV;* oocyte Stage 4; *ov. sp.* ovular space (space once occupied by the ovum); *res. grm. cl.*, resting germ cell; *rs. spm*, residuary sperm; *rst, spg. cl, resting* spermatogonial cluster; *spg.* spermatogonia; *spm*, spermatozoa; *sp. cyt*, spermatocyte; *spm. td.*, spermatid; *ylk. glb*, Yolk globule; *ylk. nuc*, yolk nucleus.]

Plate 1

Fig. 1. Photomicrograph of a transverse section of the testis of *Hilsa ilisha*, in December. Note well-formed seminiferous tubules filled with resting spermatogonial clusters. Interstitial cells are present in the intertubular spaces. × 369.

2. Photomicrograph of a transverse section of the testis in January, showing the beginning of spermatogenesis. Note germ cells in a cluster, all in the same stage of activity. × 177.

3. Photomicrograph of a transverse section of the testis at the end of February, showing progress of spermatogenesis and a widening of the lumen of the seminiferous tubules. × 177.

4. Photomicrograph of a transverse section of the testis at the end of March containing large number of sperms in the lumen of the seminiferous tubules. Note partial emptying of the lumen. × 294.

5. Photomicrograph of a transverse section of the testis in April showing cessation of spermatogenesis, resting spermatogonia and a few residuary sperms being left in the tubular lumen. × 369.

6. Photomicrograph of a transverse section of the testis early in May when it is in the dormant condition. × 369.

7. Photomicrograph of a transverse section of the testis in the middle of June, showing the recommencement of spermatogenesis × 159.

8. Photomicrograph of a transverse section of the testis at the end of July, showing active spermatogenesis. Note distension of the lumen with sperms. "Syntubular" condition is evident. × 159.

9. Photomicrograph of a transverse section of the testis in the beginning of November. Note characteristic "spent" condition of the testis showing desquamated cells in the lumen of the tubules and the hypertrophy of the intertubular connective tissue. × 159.

10. A magnified photomicrograph of the above. Note a few reserve germ cells in the tubules along with necrotic cells. × 369.

PLATE 2

FIG. 1. Photomicrograph of a transverse section of the ovary in January, showing predominance of Stage II oocytes. × 75 [Horizontal black line points to "ylk. nuc." which is unlabelled here.]

2. Photomicrograph of a transverse section of the ovary in February. Note appearance of yolk in the periphiral vesicles of the oocytes. × 76.

3. Photomicrograph of a transverse section of the ovary in March, showing deposition of yolk and the empty yolk vesicles. × 76.

4. Photomicrograph of a transverse section of the ovary in April, showing a number of ova in the process of atresia and resorption and a few Stage I oocytes. × 57.

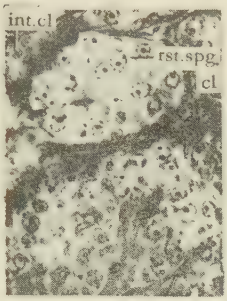
5. Photomicrograph of a transverse section of the ovary in the middle of May when it is full of Stage I oocytes. An atretic oocyte is seen in center of the photograph. × 75.

6. Photomicrograph of a transverse section of the ovary in August, showing fully mature, round ova. × 18.

7. Photomicrograph of a transverse section of the ovary at the end of October, showing follicles from which the ova have escaped. The follicular wall has become hypertrophier (the so-called corpus luteum formation), the space which lodged the ovum being still discernible. × 57.

8. A magnified photomicrograph of a part of the above showing a single empty hypertrophied follicle. × 255.

9. A photomicrograph of the transverse section of the ovary in the beginning of November, showing Stage I oocytes in the resting condition. × 72.



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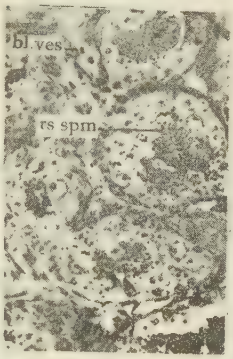

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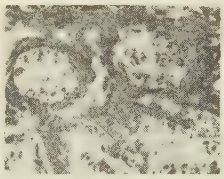

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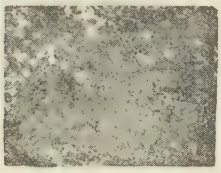

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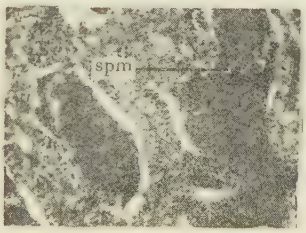

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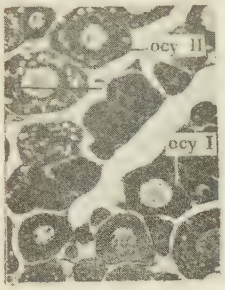
PLATE 1.



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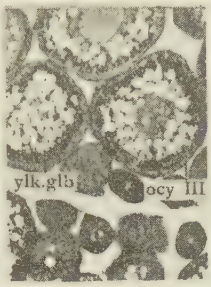

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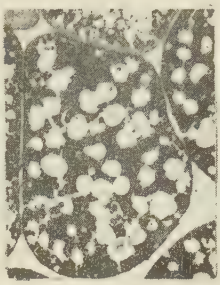

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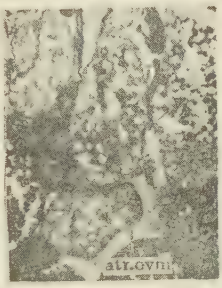

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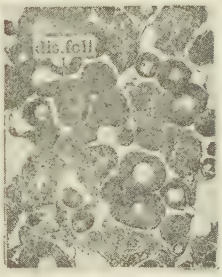

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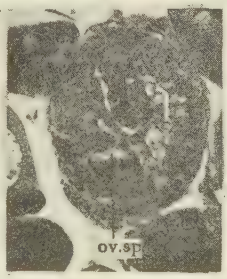

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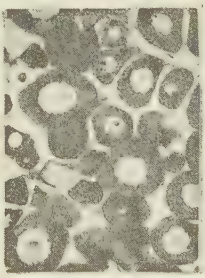

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PLATE 2.

NEW AND NOTEWORTHY PHILIPPINE MOSSES, II

By Edwin B. Bartram

Several small but interesting collections of Philippine mosses, principally by Dr. Jose V. Santos and Mr. Juan C. Pancho, have come in since the last report was made.[1] The new and previously unrecorded species detailed in the following list suggest that further explorations will expand the Philippine moss flora to a considerable extent. The well known mountain regions of Luzon continue to produce new records and extensive unexplored areas in the other islands will surely add appreciably to this rich tropical flora.

LEUCOBRYACEÆ

LEUCOBRYUM CHLOROPHYLLUM C. M.

Negros, Negros Oriental, near Dumaguete, on stump, deep woods, alt. 3,500 ft., coll. Margaret P. Mack, April 20, 1951. From Dr. Paul M. Patterson, Hollins College, Va. New to the Philippines.

The small size and slenderly pointed leaves, rugose on the back above and showing only one layer of leucocysts above and below the median row of chlorocysts clearly separates this material from any of its local allies.

POTTIACEÆ

TRICHOSTOMUM (OXYSTEGUS) BREVIFOLIUM Bartr., sp. nov.

Caespitosum, caespitibus densis, fuscescentibus, opacis. Caulis usque ad 1 cm altus, simplex vel parce ramosus, haud radiculosus. Folia sicca arcte incurva, humida erecto-patentia, 1.5–2 mm longa, e basi ovata oblongo-lanceolata, carinata, obtusa, breviter apiculata; marginibus erectis, integris; costa ad basin 45μ lata, breviter excurrente; cellulis superioribus minutis, obscuris, dense papillosis, basilaribus breviter rectangularibus, pellucidis, parietibus firmis, marginibus versus subquadratis. *Caetera ignota.*

Luzon, Tirad Pass, Sitio Sisim, Barrio Mabatano, Mt. Tirad, Concepcion, Ilocos Sur Province, through secondary forest, on rock, José V. Santos No. 5701, May 7, 1953.

[1] New and noteworthy Philippine mosses. Farlowia 1[4] (1944) 503–513.

The shorter, more bluntly pointed leaves and the shorter basal leaf cells with firm, pellucid walls will distinguish this species from *T. cuspidatum* Doz. & Molk.

MNIACEÆ

MNIUM ROTUNDIFOLIUM Bartr.

LUZON, Mountain Province, Mt. Polis, epiphyte on Rhododendron, alt. 6,155 ft., Juan V. Pancho No. 5604. New to the Philippines.

A welcome addition to the Philippine flora of a species previously known only from eastern New Guinea and the Cape York Peninsula of northern Queensland, Australia. As the sporophyte is unknown the true position of this species is still open to question.

RHIZOGONIACEÆ

NEGROS, Negros Oriental, Dumaguete, on base of tree fern in deep woods, alt. 3,000 ft., coll. Margaret P. Mack. From Dr. P. M. Patterson.

The only other local collection of this attractive little species is from Sax River, Mindanao by Williams. Its range is restricted to the Celebes and Papua, New Guinea as now known.

ORTHOTRICHACEÆ

ZYGODON REINWARDTII (Hornsch.) Al. Br.

MINDANAO, Davao Province, Mt. Apo, on tall trees, alt. 4,000 ft., Juan V. Pancho No. 2518. New to the Philippines.

The occurrence of this widely distributed species in the Philippines is welcome but hardly unexpected. Area: Alaska, Mexico, Guatemala, Costa Rica, West Indies, wide in South America, Pacific Islands, India, and Africa.

GROUTIELLA GONIORHYNCHA (Doz. & Molk.) Bartr., comb. nov.

Schlothemia goniorhyncha DOZ. & MOLK. in Pl. Jungh. 1 (1854) 358.

LUZON, Mountain Province, Mt. Polis, epiphyte, Juan V. Pancho No. 5605.

A frequent species in Luzon. Area: Khasia through Malaysia to New Guinea.

TRACHYPODACEÆ

TRACHYPODOPSIS INCLINATA (Wils.) Fleisch.

PALAWAN, Mt. Apis, west bank of Talikaigen River, L. Ebalo & H. C. Conklin No. 1383. New to the Philippines.

Readily distinguished from *T. crispatula* (Hook.) Fleisch. by the shorter leaf points and absence of basal auricles. Area: Himalaya, Java.

METEORIACEÆ

AEROBRYOPSIS LONGISSIMA (Doz. & Molk.) Fleiwch. var. GIGANTEA Fleisch.

MINDANAO, Davao Province, Mt. Apo, mossy forest, alt. 6,000 ft., Juan V. Pancho No. 2600.

Very robust, reddish brown plants in dense mass that seem to be referable to this variety.

AEROBRYOPSIS SCARIOSA Bartr.

LUZON, Mountain Province, Mt. Polis, epiphyte at 6,000 ft., Juan V. Pancho No. 3776.

The second collection of this endemic species formerly known only from the original locality on Mt. Santo Tomas.

THUIDIACEÆ

HERPETINEURUM TOCCOAE (Sull. & Lesq.) Card.

LUZON, Mountain Province, Mt. Polis, on moist soil, alt. 6,500 ft., Juan V. Pancho No. 5609.

Previously known only from Santos' collection No. 862 from Quezon (Tayabas) Province.

BRACHYTHECIACEÆ

RHYNCHOSTEGIELLA SANTOSII Bartr., sp. nov.

Autoica, gracilis, saxicola, caespitosa, caespitibus densiusculis, depressis, intertextis, lutescentibus, nitidiusculis. Caulis sat dense irregulariter ramosus, ramis circa 5 mm longis, obtusis. Folia conferta, horrida patula, late ovato-lanceolata, raptim subulato-acuminata, ad 1.3 mm longa, 0.7 mm lata; marginibus planis, ubique serrulatis; costa tenuis, ad ⅔ folii producta; cellulis breviter linearibus, 25–30μ longis, 7–8μ latis, obscuris, vix incrassatis. Folia perichaetialia interna erecto-appressa, oblongo-lanceolata, acuminata, ad 2 mm longa. Seta tenuis, 18 mm longa, ubique humiliter papillosa; theca suberecta, oblonga, deoperculata 2 mm longa; operculum oblique conico-rostratum.

LUZON, Barrio Agawa, Besao, Mountain Province, Bontoc Subprovince, Sitio Banalaw, clearing along edge of primary forest, on rock, alt. 4,000 ft., Jose V. Santos No. 5379.

The crowded, broader leaves, shorter leaf cells and weakly scabrous seta adequately separate this species from *R. Edanoi* Broth. Superficially the plants are suggestive of *Campylium*.

ENTODONTACEÆ

ENTODON RAMULOSUS Mitt.

LUZON, Mountain Province, Barrio Agawa, Besao, pine forest, Sitio Masini, on rock, alt. 3,300 ft., Jose V. Santos No. 5474. Mountain Province, Mt. Polis, epiphyte on Quercus, alt., 6,155 ft., Juan V. Pancho No. 5603.

These collections compare favorably with Elmer's No. 22268 from Camp Stotsenburg (Mt. Pinatubo) but unfortunately are without fruit. Until fertile plants are available the status of this species in the local flora must remain doubtful.

SEMATOPHYLLACEÆ

HETEROPHYLLUM BIPINNATUM Bartr., sp. nov.

Dioicum, dense caespitosum, caespitibus depressis, pallide flavescentibus, nitidiusculis. Caulis prostratus, usque ad 5 cm longus, sat dense bipinnatim ramosus, ramis late patulis, circa 1 cm longis, flexuosis, dense foliosis. Folia caulina falcata, 1.2 mm longa, e basi circa 0.6 mm lata sensim acuminata, ecostata; marginibus planis, superne minutissime denticulatis; cellulis laminalibus anguste linearibus, sinuosis, alaribus numerosis, hyalinis, oblongis, vesiculosis, saepe decurrentibus. Folia ramea multo minora, erecto-patentia, 0.75 mm longa, ovata, concava, breviter acuminata, superne minutissime denticulata, cellulis alaribus paucis, hyalinis. *Caetera ignota.*

LUZON, Mountain Province, Bontoc Subprovince, Barrio Agawa, Besao, primary forest, alt. 5,500 to 5,800 ft., on tree root, near summit of Mt. Sisipita, Jose V. Santos No. 5578.

Sharply distinct from *H. Santosii* Bartr. in the subentire leaves and the alar cells of the stem leaves hyaline, thin-walled and decurrent.

NATURE OF PHILIPPINE PORCELAIN, II

PHILIPPINE DINNERWARE

By Gil O. Opiana

National Institute of Science and Technology, Manila

and

Fujii Kanekoto*

ONE PLATE

In Part I of this paper,[1] the chemical analyses of some probable ceramic raw materials available in the Philippines, including some of foreign origin, were given. Mention was also made there of the existence of local deposits available for porcelain making.

In this paper, the possibility of producing in the Philippines a type of porcelain dinnerware made from local materials is brought out.

Analyses, test and comparisons were made between the various types of clays, spars, and kaolins. Korean kaolin, which is employed in various laboratories and industries in Japan, was used as reference standard for comparison. The tests included molding quality, air shrinkage, fire shrinkage, water of plasticity and color of tests pieces at Cone 10 (oxidizing atmosphere). The fire test was carried out in a coal-fired kiln and the temperature was raised to Cone 10 according to

Table 1.—*Chemical analysis of samples of Philippine porcelain materials.*

Samples	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	K_2O	Na_2O	Ign. loss	Remarks
Pansol	44.92	36.00	1.60	------	------	------	2.39		15.09	----------
Tiwi-spar	62.16	18.24	3.20	------	4.15	2.01	4.22	2.90	5.01	----------
Baruen spar	68.86	18.16	0.24	------	1.01	0.39	0.42	7.92	0.30	----------
Iloilo	61.50	27.76	------	------	0.46	0.37	1.41		8.20	----------
Bayas	64.98	22.91	1.79	------	------	0.56	0.56	1.62	0.31	----------
Fornier	71.34	19.91	1.23	------	------	0.86	0.99	1.24	4.34	----------
Opao	63.74	19.21	2.91	------	0.28	0.93	2.49	1.69	5.38	----------
Tiwi white	55.09	24.58	6.60	0.78	1.07	0.24	0.47	1.09	9.90	MnO–0.21
Tiwi nag	41.99	35.04	1.80	1.34	0.11	0.17	0.89	0.34	15.14	SO_3–3.15
Bacolod	62.08	26.00	0.28	0.95	0.16	0.06	0.11	0.16	10.31	----------
Angat spar	70.62	17.02	1.20	------	2.03	0.30	4.47		4.36	----------
Angat silica	82.94	9.38	2.42	------	------	0.43	1.09		3.74	----------
Angat clay	70.90	18.19	2.09	------	0.25	0.65	1.21		6.71	----------
Korea	44.46	37.02	0.21	------	0.53	0.25	0.52	0.36	12.34	Standard

* Formerly Director of Government Research Institute for Ceramics, Kyoto, Japan.

[1] Philip. Jour. Sci. 80 (1951) 1–10.

standard practice of firing. Cone 10, which is the maximum temperature employed in firing Japanese dinnerware products, was used. The test samples were prepared in accordance with the standard method and procedure. Table 1 gives the chemical analysis of the samples tested and Table 2 shows the results of some physical tests made on them.

TABLE 2.—*Molding and firing tests of some Philippine porcelain materials.*

Samples	Molding tests			Firing tests—Cone 10 (oxidizing atmosphere)		
	Water of plasticity	Comparison with Korean kaolin	Air drying shrinkage	Physical effects	Color	Fire shrinkage
			Per cent			*Per cent*
Pansol	35.1	Superior	5.8	Not vitrified	Lemon yellow with spots	20.2
Tiwi spar	2.0	Inferior	1.9	Melted	Dark brown	-------
Baruen spar	-------	------do	-------	------do	Silver gray with spots	-------
Iloilo	35.5	Same as Korean	8.1	Not vitrified	Light lemon yellow	7.7
Bayas	37.5	Superior	12.9	------do	Light yellow	6.3
Fornier	23.8	Inferior	0.7	Vitrified	Light yellow with spots	8.1
Opao	22.0	------do	1.3	Not so much vitrified	Silver gray brown to black	6.9
Tiwi white	27.1	Superior	9.7	------do	Light brown	10.7
Tiwi nag	24.0	Same as Korean	6.8	------do	Ash gray little spots	20.1
Bacolod	28.0	Superior	6.1	Not vitrified	Light silver gray	10.3
Angat spar	-------	Inferior	-------	Melted	Silver gray	-------
Angat silica	-------	------do	-------	Not vitrified	Brown to black	1.0
Angat clay	32.6	Same as Korean	6.2	------do	Brown to yellow	7.5

The results obtained for each sample are further described below:

Pansol.—The clay is plastic and can be shaped alone. This is kaolin and a good material for porcelain making.

Tiwi spar.—Lacking in plasticity, this completely melts to a dark tea-colored piece. It can be utilized as raw material for colored pottery.

Baruen spar.—As a spar, it can be used in glazing and is better material for colored pottery than Tiwi spar.

Iloilo.—Because it cannot be shaped alone, the addition of a binder is necessary to work on this. A good material for porcelain manufacture, it needs washing to remove some hard impurities.

Bayas.—This clay has good plasticity. A fired piece shows brown spots. It requires washing to remove its impurities. It can be used as a material for colored pottery.

Fornier.—Its poor plasticity, makes it hard to shape alone. A fired piece is silvery gray in color. It can be used for colored potteries.

Opao.—It has poor plasticity. It contains plenty of black spots which have to be removed. It has to be washed to utilize it in colored pottery pieces. A fired piece has an ash gray color.

Tiwi white (with streak).—Its plasticity is good, but it has plenty of iron. The clay could be used for colored potteries.

Tiwi nag.—This has poor plasticity. It contains plenty of brown spots. It can be used in colored pottery wares.

Bacolod.—This has good plasticity. It can be considered as a kaolin and is good for porcelain making.

Angat spar.—It lacks plasticity, and melts at Cone 10. The fired piece is silvery gray. The spar can be used as a material for colored pieces.

Angat silica.—This is the same as Angat spar in color of the fired piece. It is partly vitrified. It can be used to correct deficiency in silica in bodies and in glazes.

Angat clay.—This has good plasticity. The fired piece is light brown and somewhat vitrified. It can be used for colored pottery wares.

The above shows that while plenty of local raw materials for colored wares exists, only three (Iloilo, Bacolod, and Pansol) could be used for porcelain manufacture. In view of these findings, further tests were carried out to find the possibility of making a certain type of colored ware using various materials. For this purpose, a Japanese body mixture as a base was used. This is the Noritake dinnerware body mixture,[2] which is made up of feldspar, 20 per cent; liparate, 40 per cent; kaolin, 25 per cent; and kibushi, 15 per cent. The chemical analysis of these raw materials is given in Table 3, while the calculated chemical composition of the compounded dinnerware body based on this proportion is shown in Table 4.

The calculated ceramic formula[3] of the body is given below:

$$\left.\begin{matrix} CaO\ —0.210 \\ MgO—0.134 \\ K_2O\ —0.346 \\ Na_2O—0.310 \end{matrix}\right\} \left.\begin{matrix} 2.92\ AL_2O_3 \\ 0.04\ Fe_2O_3 \end{matrix}\right\} 12.52\ SiO_2$$

Ratio of Al_2O_3 to silica is 1:4.26.

[2] Calculated by C. Erfe, ceramist, Ceramic Section, National Institute of Science and Technology, using Washington's method as modified by Wilson.

[3] Ceramic calculations by I. Andrews.

TABLE 3.—*Chemical composition of Japanese dinnerware materials.*

Materials	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	K_2O	Na_2O	Loss Ign.
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Feldspar	65.13	19.11	0.34	--------	0.87	0.43	7.10	6.04	1.12
Liparate	77.44	14.80	0.38	trace	1.84	0.46	1.92	0.52	3.01
Kaolin	43.79	40.14	0.53	0.33	0.17	0.61	0.54	0.89	14.10
Kibushi	55.17	30.72	0.92	0.03	0.30	0.21	2.98	0.35	9.40

TABLE 4.—*Chemical composition of the Japanese dinnerware body compared with the calculated chemical composition of the Philippine dinnerware body.*

Constituents	Japanese body *Per cent*	Philippine body *Per cent*
SiO_2	67.14	66.78
Al_2O_3	26.45	26.70
Fe_2O_3	0.57	1.82
CaO	1.05	0.67
MgO	0.48	0.30
K_2O	2.89	1.20
Na_2O	1.71	1.65

Four raw materials were employed in the compounding of the Japanese dinnerware body based on the ceramic formula. Also, the greater bulk was 40 per cent liparate which was a potter's stone, rich in silica and quite low in alumina. No free quartz or silica was added to the compounded body and liparate became the source for silica in the final mixture. With this formula as the guide and as a starting point, a Philippine dinnerware body mixture composed of local raw ceramic materials, which were tested as given in Table 2, was compounded.

After a series of trials and calculations, the following mixture was selected for the tests: Angat spar, 30 per cent; Angat silica, 8 per cent; Angat clay, 25 per cent; and Pansol clay, 37 per cent. The chemical composition of these materials is given in Table 1 and their calculated composition is shown in Table 4.

The ceramic formula calculated from the chemical composition is given below.

$$\left.\begin{matrix} CaO\ —0.204 \\ MgO—0.127 \\ K_2O\ —0.216 \\ Na_2O—0.452 \end{matrix}\right\} \left.\begin{matrix} 4.46\ Al_2O_3 \\ 0.194\ Fe_2O_3 \end{matrix}\right\} 19.95\ SiO_2$$

Ration of Al_2O_3 to SiO_2 is 1:4.48.

A critical examination of the ceramic formula of the prepared body from local materials and that of the Japanese body shows:

1. Both bodies have approximately the same amount of CaO and MgO.
2. The amount of iron (Fe_2O_3) in the Philippine body is around four and one-half times that of the Japanese, hence its color.
3. In K_2O, the Japanese body is about one and one-half times that of the Philippine body and in Na_2O the Japanese body is about sixty-eight per cent of the Philippine body.
4. The ratio of Al_2O_3 to SiO_2 in the Japanese body is 1:4.26, and that of the Philippine body is 1:4.48. Variation in the amount of alumina is from 2.92 to 4.46; in silica from 12.52 to 19.95. The range is rather wide and seems to be favorable to Philippine materials.
5. If iron (Fe_2O_3) is included in the fluxes, the Japanese body has a total flux of 6.7 per cent while the Philippine body has 5.62 per cent.

The compounded Philippine body is somewhat similar in chemical composition to that of Noritake china body, given in Table 4. It has a good air-drying quality, and it could easily be molded and jiggered. It is also plastic. Cups and saucers and small plates made with it were biscuited at 850°C. The biscuits were light cream in color. They were glazed with clear standard glazes, which produced shiny pieces having white ash color. Such standard glazes have the following formulas:

a. $\left.\begin{matrix}0.4\ K_2O \\ 0.6\ CaO\end{matrix}\right\}$ $0.5\ Al_2O_3.\ 4\ SiO_2$

b. $\left.\begin{matrix}0.2\ K_2O \\ 0.8\ CaO\end{matrix}\right\}$ $0.35\ Al_2O_3.\ 4\ SiO_2$

c. $\left.\begin{matrix}0.3\ K_2O \\ 0.5\ CaO \\ 0.2\ ZnO\end{matrix}\right\}$ $0.35\ Al_2O_3.\ 2.8\ SiO_2$

d. $\left.\begin{matrix}0.3\ K_2O \\ 0.2\ Na_2O \\ 0.2\ MgO \\ 0.3\ CaO\end{matrix}\right\}$ $0.7\ Al_2O_3.\ 5.9\ SiO_2$

All the glazes with the above formulas fused and matured at cone 10. There were no crazes after one and one-half years. In order to find out whether one firing could be applied to the prepared body, raw cups were glazed and fired. The resulting pieces were also good. The glazed pieces which had light ash color were similar to those imported from Hongkong vulgarly called *pingan bastos*. They had a good bell sound. (Plate 1)

TABLE 5.—*Mineral composition of Japanese and Philippine dinnerware bodies.*[4]

Constituents	Japanese body *Per cent*	Philippine body *Per cent*
K-spar	15.80	5.56
Na-spar	13.40	10.48
Ca-spar	4.70	4.17
Clay	43.00	45.15
Silica	21.80	32.40
Magnesite	0.90	0.49
Limonite	0.45	1.46
Total	100.05	99.71

Table 5 shows the mineral composition of the compounded bodies calculated from their chemical composition.

Examination of the mineral composition shows:

(1) There are very wide differences in percentages in the case of spars. The Japanese body has more of each of the spars than the Philippine. Small deviation was noted in the case of lime spar.

(2) The variation in clay is not so marked. The Philippine body has a slightly higher percentage than the Japanese.

(3) A very noticeable difference was noted in the case of silica. The Philippine body is quite high in this mineral.

(4) The limonite in the Philippine body is about three times that of the Japanese, hence its color.

(5) Although the percentage of magnesite is low, the Japanese body has nearly twice as much magnesite as the Philippine. From the standpoint of general ceramic composition, the Philippine body is somewhat refractory. In round figures, the Philippine body is composed of 20 per cent spar, 45 per cent of clay, and 35 per cent silica; and the Japanese body is made up of 30 per cent spar, 45 per cent clay, and 25 per cent silica.

SUMMARY

1. The chemical analysis of the different local ceramic materials including some of their physical properties in comparison with that of Korean kaolin are given.

2. Some of the local clays compare favorably with Korean kaolin in molding quality while others are inferior.

3. Some of the samples melted, others somewhat vitrified, while others were not vitrified at cone 10.

[4] From an unpublished report on file in the Records Section, National Institute of Science and Technology.

4. The ceramic formula of Japanese dinnerware body and that of a possible Philippine body were compared.

5. The ratios of Al_2O_3 and SiO_2 in Philippine and Japanese bodies are 1:4.48 and 1:4.29, respectively.

6. Using standard glazes maturing at Cone 10, the Philippine body could be glazed after biscuiting or even before firing.

7. By compounding different local ceramic materials a dinnerware body of certain type and color was made. Owing to the presence of iron oxide, the finished product was white ash in color.

8. The mineral constituents of both Japanese and Philippine dinnerware bodies were also compared. Differences were observed in silica, in total spars, and in limonite.

9. The results show the possibility of making colored dinnerware and allied products from local ceramic materials.

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ILLUSTRATIONS

PLATE 1

FIG. 1–4. First trial
5–8. Final mixture
9. Red body glazed under atmosphere.

PLATE 1.

DISEASES OF THE CHORION

By Honoria Acosta-Sison
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ONE PLATE

Hydatidiform mole is the conversion of a great part or all of the chorionic villi into cystic structures. There are 2 varieties—the false or transitional mole and the true mole.

Chorioadenoma destruens is a chorioma in which a mole syst or chorionic villus along with trophoblasts have invaded or penetrated the myometrium.

Syncytial endometritis or syncytioma consists of a group of syncytial cells that have infiltrated the myometrium causing marked inflammatory reaction and perhaps necrosis.

Choriocarcinoma is the most malignant trophoblastic tumor composed of both Langhans and syncytial cells usually with a predominance of the former.

HYDATIDIFORM MOLE

Hydatidiform mole is an abnormal pregnancy resulting from a pathologic ovum wherein there is proliferation of the epithelial covering of the chorionic villi and dissolution and cystic cavitation of their avascular stroma.

The villi that had been converted into molar cysts look very much like bunches of grapes connected with one another by a tenuous tissue; the cysts varying in size with a diameter from 0.5 mm to 3 cm.

Microscopically, the mole cyst shows hydropic degeneration of the stroma and proliferation of the Langhans and syncytial cells.

Though about 10 per cent of cases of hydatidiform mole are complicated by chorionic malignancy and that over 50 per cent of cases of choriocarcinoma are preceded by hydatidiform mole, according to Hertig, the process of mole formation is not "of a degenerative nature but a physiologic one whose basic principle rests upon the disappearance of a functional fetal blood supply from chorionic villi whose trophoblast is still living."

The mole cysts begin to be formed, according to Hertig, from the third to the fifth developmental week when normally the fetochorionic circulation becomes established. But since the

fetus is absent, or defective, or had an early death, there occurs no chorionic circulation and this results in the dissolution of the mesodermic core. The trophoblasts on the other hand, being nourished by the maternal blood in the intervillous spaces, continue to live and are functionally active in secreting and transferring substances from the maternal blood into the interior of the villi. There being no chorionic circulation, their liquid products increasingly accumulate within the villi causing their distention and formation into mole cysts.

According to the chemical studies of Mckay, et al. mole fluid is an interstitial fluid essentially similar in composition to other environmental fluids such as ascitic and edema fluid and that of the normal tendon. It, however, has lower chloride content than the ascitic or edema fluid. It has lower sodium, chlorides, calcium, protein, and iron, but higher phosphorus, phosphates, alkaline and acid phosphatase, and water content than the serum. Mole fluid has the same concentration of ascorbic acid and non-protein nitrogen as that found in the serum. The mole fluid was found also to contain albumin and/or protein, and protein-bound iodine presumably thyroxin. But it has no cholesterol.

The higher concentrates of amino acids in the mole fluid show the activity of the trophoblasts. The trophoblasts allow the passage of small amounts of normal serum albumin, thyroxin, and glucose at a slow rate.

Alter and Cosgrove distinguished between a false or transitional mole and a true mole. In false or transitional mole the changes are not neoplastic but degenerative. They are benign and usually occur with a fetus as part of a uniovular conception.

The writer observed among 317 cases of hydatidiform mole in single pregnancy, 2 cases of transitional mole with living fetuses which aborted respectively at 4 and 5 months gestation. The peculiarity noted in the cystic changes was that the cysts were quite large having a diameter of 1 to 1½ inches. Within 3 years afterwards both mothers delivered spontaneously term babies.

Berry reported a case of transitional mole with a sixteen-week well formed but nonviable fetus. Bowles reported in 1943 a living 32-week fetus with 75 per cent cystic degeneration of the placenta. This fetus is now 15 years old. Bowles

cited 17 other cases in the literature of fetuses whose placentæ showed extensive molar degeneration.

Apparently, in all the above cases the cystic degeneration of the chorionic villi did not occur until after the fetus was well formed. And the fetus was able to survive so long as it could get enough blood supply through the remaining healthy villi.

The true mole as described by Marchand in 1895 is characterized by neoplastic proliferation of the chorionic epithelium. According to Alter, the proliferation of the trophoblasts begins before the development of the chorionic blood vessels and is progressive in character. The true mole is never accompanied by a fetus except in biovular twin pregnancy where one twin is a normal fetus with its normal placenta, and the other a true hydatidiform mole. Acosta-Sison had reported 3 cases of such twins. In two cases of biovular twin pregnancy, one was a hydatidiform mole, and the other a 2-month fetus, and a 4-month fetus, respectively, with their corresponding placenta. The mother of the 4-month fetus had to be hysterectomized one week after abortion, for she developed uterine chorioepithelioma. She finally recovered.

In the third case of biovular twins the pregnancy reached 7 months. The patient first expelled the hydatidiform mole, then delivered a 7-month alive fetus followed by its normal placenta. A few minutes after expelling the placenta, the patient complained of headache, then became unconscious. This was soon followed by death. Autopsy revealed metastatic chorioepithelioma in the vagina, lungs, and brain with cortical hemorrhages.

Since over 50 per cent of choriocarcinoma is the sequel or the accompaniment of hydatidiform mole, an attempt was made to find in the mole curettings some signs which may indicate actual or future malignancy. The writer found that when most of the cysts are very tiny, 1 to 2 mm in diameter, and specially if they are accompanied by solid placentalike tissue, a subsequent malignant outcome is to be feared.

Hertig and Sheldon tried to correlate the degrees of hyperplasia and the presence of anaplasia in the trophoblast with the outcome of malignancy. They found that in what they now call Grade 3 (formerly Grade 6) where there was exuberant trophoblastic growth and marked anaplasia, malignancy was the outcome. Such a finding does not mean that apparently

benign moles will not be followed by chorionic malignancy.

The value of their work is to make the clinician watchful for malignancy not only in all cases of hydatidiform mole but specially in those cases where there is much proliferation of the trophoblasts.

Biologically, the hydatidiform mole is accompanied by high values of chorionic gonadotropin in the blood serum and urine. Chorionic gonadotropin is also found in the spinal fluid. It is said to be secreted by the Langhans cells.

In a study on the determination of chorionic gonadotropin in the urine of 51 cases of hydatidiform mole, Cajipe and de la Paz of the North General Hospital, Manila, found that 30 cases showed from 800,000 to 2,000,000 I.U. The high values of chorionic gonadotropin showed the activity of the Langhans' cells.

According to Delfs, the early days of normal pregnancy is accompanied by high values of chorionic gonadotropin. The peak is reached at about 60 days after the last menstruation and may reach as high as 600,000 units. A broader peak involving titers above 50,000 I.U. extends from 45 to 85 days. After this time the titer decreases rapidly. After 100 days the titer is rarely over 20,000 I.U. and varies around a mean of 5,000 I.U.

So that, according to Delfs, to diagnose hydatidiform mole by bioassay before 100 days after the last menstrual period is not tenable. According to Hobson, the chorionic gonadotropin values both in normal pregnancy and hydatidiform mole are extremely variable. His figures for chorionic gonadotropin in 23 cases of hydatidiform mole was from 3,000 to 30,000 I.U.; in 6 cases, 300,000 I.U.; in 4 cases, 368,000 to 1,950,000 I.U. During normal pregnancy, he found the peak of chorionic gonadotropin to be 450,000 I.U. excreted during the first 4 months of pregnancy. But in one twin pregnancy the value of chorionic gonadotropin at the 14th week was between 1,200,000 and 1,500,000 I.U.

Ovarian changes associated with hydatidiform mole.—In many cases of hydatidiform mole, one or both ovaries show theca lutein cysts which may or may not be palpable. They result from the chorionic gonadotropin stimulation of the lutein elements. The fluid of the lutein cysts is said to store chorionic gonadotropin which explains the positive reaction to pregnancy test long after the mole expulsion. Hobson

found lutein overian cysts in 23 per cent of 176 cases of hydatididiform mole. The cysts usually regress after the complete evacuation of the mole so that it is not necessary to remove them to effect cure. However, the writer has seen at least 2 cases where bilateral ovarian cysts became very much larger one to two weeks after the evacuation of the mole contents. In one case each ovary was larger than a man's head and the patient reentered the hospital because of the huge abdominal enlargement together with edematous extremities that developed 10 days after mole evacuation. Panhysterectomy and bilateral salpingo-oophorectomy were performed. Only a 2 mm growth was found in the uterus. The patient recovered.

Recurrent mole.—Cosgrove, et al., cited 42 women who had recurrent moles. Of these 42 women, only 5 had normal pregnancies between the moles. To this list the authors add their case in which a mole in the first pregnancy was followed by 3 normal pregnancies and then another mole.

The writer had found that among 155 cases of hydatidiform mole admitted to the Philippine General Hospital from January 1, 1955, to January 15, 1958, 4 or 2.58 per cent were cases of repeat moles. In 2 cases the mole occurred in succession at the third and fourth pregnancy, and 2 were interspersed respectively by one and two deliveries. Of the 4 cases of repeat mole, 3 or 75 per cent were complicated by chorionic malignancy. The type of chorionic malignancy was choriocarcinoma in 2 cases and chorioadenoma destruens in 1 case.

Tubal hydatidiform mole.—Since tubal pregnancy is possible, it is not surprising that some of these pregnancies take the form of hydatidiform mole. It is, however, quite rare. Kika and Matuda reported a case of primary tubal hydatidiform mole and cited 16 others reported in the literature. A urinary gonadotropin test of their case before laparotomy showed it to be positive in 1:5 dilution. After removal of the tube that harbored the mole which had the size of a hen's egg, the test became negative. At the end of 2 years after the mole was removed the patient delivered a term alive baby, showing the mole to be benign.

Incidence of hydatidiform mole.—The incidence of hydatidiform mole is given independently by Eastman and Hertig to be 1 in 2,000 pregnancies. Novak gave the incidence as 1 in 2,500 pregnancies. In Brazil, Fernandez gave the inci-

dence as 1 in 1,071 pregnancies. Hydatidiform mole is then a rare condition among Europeans and Americans. This is not the case in the Orient. Hasegawa gave the incidence in Japan as 1 in 232 deliveries. Prowirohardjo, et al, of Indonesia treated 92 cases of hydatidiform mole in 3 years (1952–1954) or an average of 30 cases per year. King of Hongkong University Hospital gave an incidence of 1 in 530 pregnancies among the Chinese. The writer gave the incidence in the Philippines during the Japanese occupation in the Second World War to be 1 in 126 pregnancies but a recent survey from January 1955, to September 30, 1957, showed 141 cases of hydatidiform mole among 27,784 pregnancies giving an incidence of 1 in 200 pregnancies.

Age incidence of hydatidiform mole.—Hydatidiform mole had been found in patients as young as the age of 14 and as old as the age of 54. Gaetane and Labriola reported a case of hydatidiform mole in a 54-year-old patient who because of her age was treated with hysterectomy and bilateral salpingo-oophorectomy with good result.

Table 1 shows that the percentage incidence of hydatidiform mole steadily increase with age. After the age of 39, though the actual number of cases of mole is less, its proportion with other pregnancies is much higher.

TABLE 1.—*Percentage incidence of hydatidiform mole among women who became pregnant within a certain age period, admitted to the Philippine General Hospital from April 6, 1945 to December 31, 1950.*
(Acosta-Sison and Baja-Panlilio)

Age	Total pregnancies	Cases of mole	Incidence of mole
			Per cent
14–19	2,841	11	0.38
20–29	14,182	63	0.44
30–39	7,514	75	0.99
40–49	1,048	25	2.38
50–54	24	3	12.5

Etiology.—Despite the existence of hydatidiform mole as early as the sixth century when Aetius wrote extensively about it, nothing has been written as to its possible cause. However, all writers say it is more common in multipara and after the age of 40.

In a survey made by the writer and Panlilio on the percentage incidence of hydatidiform mole among 1,048 pregnant wo-

men between 40 and 49 years of age, it was found that 25 or 2.38 per cent were cases of hydatidiform mole. And of the 24 women between the ages of 50 and 59 who became pregnant, 3 or 12.5 per cent were cases of hydatidiform mole. This is in marked contrast with the 0.38 per cent incidence of mole among 2,841 pregnant women who were 15 to 19 years of age.

What can be the basic cause of hydatidiform mole? And why the high incidence of mole in the Philippines and the rest of the Oriental countries? It is true that in these regions multiparity and pregnancy in the elderly are not uncommon. Birth control is not practiced. But the above data do not explain the occurrence of mole in young primigravidæ.

The writer's observations as presented at the Second World Congress in Obstetrics and Gynecology at Montreal, Canada, point to the deficient or inadequate intake of high class protein as found in meat, eggs and milk-food material by the patients in her survey. The patients subsisted mostly on rice and fish (often salted dried fish) and perhaps vegetables and fruits. Indulgence in such a diet is due partly to the native relish for such food and mostly to the low socio-economic condition. This basic diet so deficient in high class protein is one that the Philippines has in common with the bulk of the population of the rest of the Oriental countries, such as China, Indonesia, Japan, and India. This is in marked contrast with the high class protein intake (obtained in meat, milk, eggs, and cheese) in Western countries as dictated by natural taste, habit, climate, and higher income.

That the cause is not racial is shown by the fact that hydatidiform mole among Filipinos was observed only among the poor, not among the well-to-do private patients, except during the Second World War when there was a scarcity of food and when meat cost many times over the present cost per kilo.

Among the primigravida, many of them gave the history of a previous episode of pulmonary tuberculosis or who lived with a husband or relative affected with chronic cough. It may be that a chronic disease like that of tuberculosis aggravates the subnutritional condition of the patient.

From the evidence at hand it seems that hydatidiform mole and perhaps the tendency to chorionic malignancy develops from a pathologic ovum as a result of faulty nutrition caused

by inadequate intake of high class protein. It may be observed, however, that not all the ova become affected as shown by the double ovum twins wherein one ovum may result in mole and the other a normal fetus.

Clinical course.—In the vast majority of cases, hydatidiform mole had the tendency to grow luxuriantly so that it markedly distends the uterine cavity out of proportion to the age of gestation. In a study of 140 cases of hydatidiform mole, the writer found that in 99 cases, or 70.71 per cent, the uterus was larger than it should be for the age of gestation. In 16 cases or 11.4 per cent the size of the uterus corresponded to the age of pregnancy. And in 25 cases or 17.85 per cent the uterus was smaller than it should be for the age of gestation. Many of the patients of the smaller uteri gave the history of having expelled cysts outside. King of Hongkong University Hospital, also found that 70 per cent of his cases of hydatidiform mole showed the uterus to be larger than it should be for the age of pregnancy.

Sooner or later parts of the mole become separated from their site of attachment so that uterine bleeding occurs from 1 to 6 months after the last menstruation, but most often uterine bleeding occurs at the third or fourth month of gestation. The bleeding may be slight at first and intermittent or continuous or it may be immediately profuse. The patient may or may not have severe morning sickness. When the uterus attains the size of five and one-half or more months of pregnancy regardless of the age of gestation, hypertension followed later by other signs of toxemia may supervene. In true mole there is no fetus in single pregnancy. The patient may complain of weakness and pallor because of blood loss; she may also have fever together with an increased pulse rate.

Though the mole may be expelled spontaneously after several episodes of bleeding, its total or complete expulsion seldom occurs spontaneously, so that remnant cysts give rise to the continuation or recurrence of bleeding. Severe hemorrhage may occur at the time or immediately after the expulsion of the mole so that the patient may succumb from shock due to hemorrhage.

In benign mole, provided it is completely evacuated from the uterus and adequate blood transfusion is given, and provided it is not complicated by chorionic malignancy, complete recovery is to be expected.

Diagnosis.—There is no question that the presence of cysts either expelled at home or discovered on vaginal examination constitutes a positive diagnosis.

In the absence of visible cysts, can the determination of the chorionic gonadotropin *per se* establish the diagnosis?

Since according to Hobson the value of chorionic gonadotropin both in normal pregnancy and hydatidiform mole is variable and since high values of C.G. are found in normal pregnancy up to 100 days after the last menstruation, it seems the determination of chorionic gonadotropin values alone cannot be relied upon to make the diagnosis. The finding of Cajipe and de la Paz in 2 cases of mole to have only 20,000 I.U. of chorionic gonadotropin and the finding of Hobson of 1,500,000 I.U. in a normal twin pregnancy at the 14th week of gestation show how equivocal are the values of chorionic gonadotropin for making positive diagnosis. The bioassay should always be related to the history and clinical findings. In many cases of hydatidiform mole, the spinal fluid is positive to the frog test. In some cases, the spinal fluid is negative to the frog test.

King, of Hongkong University Hospital, based his diagnosis of hydatidiform mole on the following points: (*1*) The size of the uterus is out of proportion to the duration of pregnancy. This was found in 70 per cent of his cases. (*2*) Bloody discharge either continuous or intermittent, often brownish rather than red. (*3*) Absence of fetal parts by palpation or by x-ray examination. (*4*) Coexistence of toxemic symptoms. (*5*) Positive biologic test of the urine in 1:50 dilution or higher with a rising titer. His average time in making the diagnosis was between 3 and 4 days and in doubtful cases 10 to 14 days or longer.

Hertig is of the opinion that though a mole may be suspected on clinical grounds (bleeding, disproportionately enlarged uterus, abnormally high chorionic gonadotropin hormone titers, enlarged cystic ovaries and absence of fetal heart tones), the diagnosis rests "only on the presence of characteristic vesicles."

Bayan and Apelo based their diagnosis on the appearance of "honey-combed" appearance of molar cysts by x-ray of the uterus after instilling 25 to 50 cc of Hypaque into the uterine cavity.

It appears that in the experience of others unless the patient gives a history of having passed mole cysts or shows the cysts on vaginal examination, the diagnosis is not made until after 3 to 7 days or even longer from the time the patient is first seen. This delay is attributed to the time consumed in the performance of the quantitative biologic test and the necessity of eliminating the presence of the fetal skeleton by x-ray.

The writer's experience among 163 cases (out of a total of 318 cases) which happened to show the typical signs and symptoms of hydatidiform mole in a uterus enlarged to the size of 5 or more months pregnancy was different. The diagnosis of mole was made within 10 to 15 minutes after seeing the patient for the first time without the aid of either biologic test or x-ray picture of the uterus to eliminate the presence of the fetal skeleton. It is true all these 163 patients happened to show findings that make one suspect hydatidiform mole. All entered the hospital for the same sympton, namely, uterine bleeding. A few complained of severe morning sickness; the majority did not.

The following findings made us highly suspicious of mole:

1. Uterine bleeding which varied in amount, color and continuity was present. Usually the first episode of bleeding was slight. In some it was only in the form of spotting, but in a few it was immediately profuse. The bleeding was continuous in some, though in most instances, it was intermittent with the tendency to increase in amount in subsequent attacks. The color of the blood was pinkish in some, reddish in others, but in many it was dark-colored. The bleeding manifested itself at the second, third, or fourth month after the last menstruation.

2. The uterus was found to be larger than it should have been for the age of gestation which varied from two to four months. The size of the uterus was that of 5 or more months of pregnancy. No fetal parts could be palpated. The uterus was one uniformly consistent solid boggy mass which was soft when relaxed but firm when contracted. No amniotic fluid could be detected. There was no history of fetal movements.

3. The attempt to palpate for ballotable or irregular mass during vaginal examination which might have indicated the

presence of the fetus gave a negative finding. What was felt in most patients was fullness of part or the whole lower uterine segment. On speculum examination, the cervix was either closed or slightly open.

With the above findings, the following procedure was made to establish the diagnosis. After exposing the cervix with the bivalve speculum and painting it with merthiolate or mercurochrome or tincture of zephiran, the uterine sound was gently introduced into the cervical canal along its axis and upward into the uterine cavity. In mole, the sound goes up to 10 or more centimeters easily along the mid-line without meeting the slightest obstruction. To clinch the diagnosis the uterine forceps was introduced into the cervical cavity and after passing beyond the internal os, its tip was made to clip a little tissue. This invariably brought out molar cysts. In very definite cases where there was bulging of the lower segment, the uterine forceps was introduced without the precedent use of the uterine sound and made to clip a little tissue above the internal os.

With the above method, the positive diagnosis of hydatidiform mole was made within 10 to 15 minutes after seeing for the first time 163 patients who had uteri the size of 5 or more months pregnancy.

When the size of the uterus is that of 4-month pregnancy or less, even though the uterus contains molar cysts, the uterine sound cannot be used as a method of diagnosis. For even in normal pregnancy when the *decidua capsularis* has not yet firmly united with the *decidua vera* the sound will go up. It is then that the quantitative determination of the chorionic gonadotropin will be of value in making the diagnosis provided it is done 100 days after the onset of the last menstruation.

False passage of sound.—It is possible for the sound to go up in the presence of missed abortion where the fetus is macerated and the membranes are nonresistant. The sound may go up also in the presence of *ablation placentæ* where the separation of the placenta together with the membranes permits the passage of the sound. But in both of these conditions where the size of the uterus is of 5 or more months of pregnancy, there should be a history of fetal movement and the examiner should be able to detect the presence of the fetus.

In case of doubt quantitative determination of the chorionic gonadotropin 100 days after the last menstruation and x-ray of the abdomen should be made.

Use of the uterine sound should take place in the hospital, never in the doctor's office or in the patient's home. If bleeding should occur after the introduction of the uterine sound, the vagina should be packed immediately with pressure against the cervix. This very rarely happens and never in a marked degree.

COMPLICATIONS

Hemorrhage.—Profuse hemorrhage may occur during or after the expulsion of the hydatidiform mole, and as a consequence of hemorrhage shock may supervene if the blood loss is not immediately replaced.

Uterine perforation.—This may be caused by the mole cyst (malignant mole) or by the curette. Perforation by the curette or by the mole cyst can and should be detected by the operator who should immediately perform hysterectomy. Though uterine perforation may occur while the mole is still *in situ* or during the process of curettage, most of our cases of uterine perforation occurred weeks or months after the curettage for mole. This will be discussed in the section of chorioadenoma destruens.

Toxemia.—Hydatidiform mole is said to predispose to toxemia. Page observed pre-eclampsia in 10 of 30 cases of hydatidiform mole. Chesley, Cosgrove, and Preece had one case of eclampsia among 57 cases of hydatidiform mole. At the Margaret Hague Maternity Hospital, toxemia did not occur in twenty-five women with hydatidiform mole before the fourth month of pregnancy, but the incidence of toxemia was 28 per cent of thirty-two patients with moles after the fourth month.

In a study of 85 cases of hydatidiform mole for the purpose of finding the relationship of mole and toxemia, the writer found that 31 or 36.47 per cent showed mild toxemia in the form of hypertention ranging from 130/80 to 200/100 mm Hg. Ten cases or 11.76 per cent showed besides hypertension, albuminuria, cylindruria, and red blood cells in the urine. Two of the cases had no convulsion but had coma before death. At autopsy, both showed the typical focal hemorrhagic necrosis of the liver found in eclampsia. And one of them showed

cerebellar hemorrhage. These two are the only cases of fatal toxemia observed so far among 360 cases of hydatidiform mole.

The symptoms of toxemia in the form of hypertension developed not in accordance with the age of gestation but in accordance with the rapidity of the growth in size of the uterus regardless of the duration of pregnancy. Thus, all the hypertensives had uteri whose fundus was at or above the level of the umbilicus even though the age of pregnancy was only two to three months. In 84 per cent of the normotensives, the height of the fundus of their uteri was only from 6 cm above the symphysis to 2 cm below the umbilicus even though the age of pregnancy was as advanced as 4 months.

Chorionic malignancy.—In the study of 141 cases of hydatidiform mole admitted to the Philippine General Hospital from January 1, 1955 to September 30, 1957, the writer found that 13 cases or 9.21 per cent were complicated by chorionic malignancy. The type of chorionic malignancy as diagnosed by the microscope was choriocarcinoma in 7 cases and chorioadenoma destruens in 6 cases. In another study on chorionic malignancy, the writer found that in 6 cases, metastatic choriocarcinoma was coexistent with the hydatidiform mole *in situ*.

Prognosis.—The prognosis is good provided the mole is evacuated early and completely, and provided there are no complications.

Mortality.—According to Mathieu, before 1930 when early diagnosis of hydatidiform mole could not be made, the mortality was approximately 12 per cent. After 1930, the mortality was reduced to 2 per cent. De Lee and Greenhill had only 1 death and it was due to sepsis among 42 cases of hydatidiform mole.

Among 141 cases of hydatidiform mole studied by the writer, 8 died giving a mortality of 5.06 per cent. The cause of death in 3 cases was acute hemorrhage. One of these cases lost 3,500 cc of blood before and during the process of evacuation of the mole. At autopsy, besides the acute anemia, metastatic choriocarcinoma was found in the lungs. In one case, the site of hemorrhage was the right broad ligament, right parametrium, posterior wall of the bladder and upper part of the vagina where the mole cysts had invaded and wherein they had greatly multiplied themselves. One case of hemorr-

hage died from lower nephron nephrosis due to blood transfusion reaction. Five cases died from metastatic choriocarcinoma. One of these cases, the primary growth in the uterus was chorioadenoma destruens.

Treatment.—Every patient with hydatidifrom mole should be examined for the presence of metastasis in the vulva, vagina, or lungs. In the presence of metastasis, panhysterectomy with the mole *in situ* and the surgical removal of the accessible metastasis should be performed. Metastasis in the lungs should be immediately treated by deep x-ray. The general condition of the patient should be supported by venoclysis and adequate amount of blood transfusion.

If the patient is young, has few living children, and there are no metastases, the mole should be evacuated completely from below by dilatation and curettage. After curettage, measures should be taken to promote contraction of the uterus by oxytocics (pitocin and ergotrate or methergin) and ice cap over the lower abdomen. I have found uterine packing (applied snugly but not too tightly) reinforced by vaginal packing to be of value. These should be removed after 24 hours. In the presence of infection, antibiotics should be given. Vitamins together with iron preparations are helpful in promoting early recovery. The curetted tissue next to the uterine wall should be examined microscopically for possible malignancy.

If the patient is 39 years old and has many living children, it is safer for the patient even in the absence of metastasis to perform hysterectomy with the mole *in situ.* Adequate blood transfusion should always be given in accordance with the need of the patient. The writer has seen a few cases of fatal chorionic malignancy as a complication of an apparently benign hydatidiform mole in women aged 38, 39, 40 or more years.

Follow-up.—Every case of hydatidiform mole should be followed for signs of the presence of chorionic malignancy which may be located in the uterus, on adnexa, or broad ligaments, or the vagina or vulva, or the lungs. This can be done by noting the presence of bleeding, which normally should be absent 7 to 8 days after the molar evacuation, the size of the uterus which should be completely involuted by the end of the fifth week after mole evacuation, the presence of a pelvic mass or purplish nodule in the vagina, by the x-ray examination of

the lungs for metastasis and by examining the urine for chorionic gonadotropin. As a rule, the frog test of the urine is negative for chorionic gonadotropin within one or two months after the curettage of hydatidiform mole.

The frog test of the urine even though it be negative for chorionic gonadotropin for 4 consecutive months following mole evacuation is not an assurance of the absence of chorionic malignancy. The test should be done monthly for at least 6 to 12 months. The writer observed a 24-year-old G_3 P_2 whose frog test of the urine for 4 consecutive months following the curettage of a benign mole was consistently negative for chorionic gonadotropin. Menstruation was normal, of 3-day duration, and came monthly. There was no abnormal bleeding nor abnormal pelvic mass. The uterus had undergone normal involution and was small at the end of one month. The patient said she felt well and had no complaint. On the 5th month after mole evacuation, her urine which she merely sent by messenger was found for the first time to be positive for chorionic gonadotropin. Twenty-eight days thereafter, she was brought to the hospital unconscious and with right hemiplegia. The blood pressure was normal. A few hours after admission she died. Autopsy showed a 4 by 4 cm metastatic choriocarcinoma nodule at the base of the right lung and a 5 by 5 cm hemorrhagic mass in the left parietal lobe of the brain. The internal genitalia were perfectly normal and showed no chorionic cells. Both ovaries only showed multiple small lutein cysts.

This is one of 4 cases, observed by the writer, who died from choriocarcinoma without exhibiting a primary growth in the uterus.

CHORIOADENOMA DESTRUENS

Chorioadenoma destruens (a term originating from Ewing) is applied to that chorioma wherein "one or more villi and associated trophoblasts invade the myometrium or its blood vessels or both" (Hertig).

Prawirohardjo, et al., of Indonesia, called such a chorioma "villous choriocarcinoma" and Novak would like to name it "mild variant of chorioepithelioma."

Most pathologists regard chorioadenoma destruens as a benign chorioma which very rarely metastasizes. According to Hertig, chorioadenoma destruens follows only a hydatidiform mole un-

like choriocarcinoma which may follow any type of pregnancy.

In a previous study of 18 cases of chorioadenoma destruens admitted to the Philippine General Hospital from 1950 to 1953 the writer found that all the 18 cases were the sequela of hydatidiform mole. Perforation of the uterus, which gave rise to intraperitoneal hemorrhage from 300 cc (in 1 case) to 1,500 cc, was found in three cases or in 17 per cent. Metastasis occurred in four cases or in 22.22 per cent. The site of metastasis was either the lung or the vagina in 3 cases. But in 1 case where the mole was still in situ and undiagnosed the metastasis and generalized as discovered at the autopsy.

In a subsequent study, also of 18 cases of chorioadenoma destruens admitted to the Philippine General Hospital from 1954 to 1957, 17 cases followed hydatidiform mole but 1 case was the sequela of term pregnancy. Among a total of 36 cases admitted from 1950 to 1957 this constituted an exception to Hertig's dictum that all cases of chorioadenoma destruens follows a hydatidiform mole.

Out of the 18 cases, seven or 38.88 per cent had uterine perforation which gave rise to intraperitoneal hemorrhage. Six cases or 33.33 per cent showed metastases in such regions as the lungs, parametrium, broad ligaments, vagina, mons veneris and left labium majus.

Prawirohardjo, et al., of Indonesia, found that out of 81 cases of hydatidiform mole, ten cases or 12.3 per cent were followed by chorioadenoma destruens. Five or 50 per cent of the 10 cases of chorioadenoma showed metastases in such regions as the lungs, parametrium, and peritoneum. In 1 case, the metastasis was not only in the parametrium and the lungs but also in the brain.

Wei, et al., also found that among their 12 cases of chorioadenoma destruens which followed mole, four or 33.33 per cent showed metastasis.

Delfs reported a case of "benign" mole where the cysts metastasized into the right lung, the extradual tissue of the cervical spinal cord nerve roots of the lumbar spinal cord and the liver. Despite the hysterectomy and bilateral salpingo-oophorectomy, lobectomy, the surgical removal of the extradural and nerve root metastases followed by complete x-ray radiation, the patient died 5 months after the completion of the x-ray treatment and 3 years and four and one-half months after the original mole. Death was attributed to "mole with

benign metastases." Others would perhaps diagnose this case as malignant mole which gave rise to metastases causing death.

Mortality.—The writer's combined studies comprising 36 cases showed that three or 8.83 per cent died. Among Prawirohardjo's ten cases, three or 30 per cent died. Wei, et al., had no mortality in their 12 cases of chorioadenoma destruens. The treatment carried out in all the above cases was hysterectomy. The lung metastases were treated by x-ray, except Wei's cases who were treated by nitrogen mustard and nitromin intravenously after hysterectomy. Wei treated the vaginal metastasis in 2 cases with radium; and in 1 case by surgical removal.

It appears that in the experience of the above authors chorioadenoma destruens is not an entirely benign chorioma and that Novak is justified to call it a "mild variant of chorioepithelioma," for not only it perforates the uterus giving rise to dangerous hemorrhage but it does metastasize (to as much as 30 per cent of cases) though not as early, nor as frequently, nor as rapidly and as widely disseminated as does choriocarcinoma.

Diagnosis.—The diagnosis of chorionic malignancy is made by the microscopic picture of the tissue obtained from the diagnostic curettage. This can be done if the growth is in the endometrium. However, if the growth is in the myometrium, beyond the reach of the curette, the diagnostic curettage will give a false negative result. The clinical method of diagnosis termed HBEs or the history of having expelled a product of conception, bleeding, and the enlargement and softening of the uterus, or in the absence of bleeding, the presence of an abnormal pelvic mass in the lower abdomen and positive frog test for chorionic gonadotropin may point to the diagnosis.

Prognosis.—The prognosis of chorioadenoma destruens is good provided hysterectomy is done before uterine perforation or before metastasis has occurred, or provided that the metastasis when present is either in the vagina or lungs and these are treated early.

Treatment.—Hysterectomy should be done with or without bilateral salpingo-oophorectomy depending on whether the adnexa are or are not affected. If not affected they should not be removed specially in young patients. If malignant chorionic cells cannot be entirely removed, surgery should be supplemented with x-ray radiation, or with testosterone or

methotrexate or nitrogen mustard or nitromin. These drugs will be discussed more in the management of choriocarcinoma.

Syncytioma or syncytial endometritis.—Marchand, as quoted by Novak, regarded syncytioma as "atypical form of chorio-epithelioma." Prawirohardjo, et al., designated syncytial endometritis as choriocarcinoma with predominance of syncytial cells. Hertig along with other pathologists regarded the histologic picture of syncytial endometritis to be "merely an accentuation of the morphology of the placental site. Endometrium and myometrium are infiltrated with trophoblastic cells with variable degrees of inflammation . . . The lesion is benign."

Mendoza-Guazon, a former pathologist of the University of the Philippines, reported in 1927 that our of 3,932 autopsies on women, six or 0.15 per cent were cases of chorionic malignancy. Histopathologic examination showed that three were cases of choriocarcinoma of the uterus with metastasis in the lungs and vagina. Two were cases of chorioadenoma destruens of the uterus with metastasis in the lungs in one case, and in the vagina in the other case. One was a case of syncytioma which had perforated the uterus.

In 1950 the writer sent a slide of the last curettings (tissues next to the uterine wall) of a patient of hydatidiform mole to Dr. Novak for histologic diagnosis. The patient did not return for check up until 13 months after curettage when she showed enlarge uterus of two and one-half month size and fulness of both broad ligaments. She complained of lower abdominal pain, dysuria and constipation, and slight oozing off and on fever since the curettage. Because of the presence of HBEs, she was immediately laparotomized. On opening the peritoneal cavity, the enlarged soft uterus was found to be fixed at its lower portion by a bloody mass connecting it with the intestines. On its anterior wall was a dark bloody mass the size of a large strawberry which was about to burst through the serous covering. Both broad ligaments were swollen with bloody growths. The abdominal incision was closed. The plan was to treat the patient by x-ray. The patient had 39°C after the operation. She left the hospital on the fourth day in serious condition and eventually died at home.

The histology diagnosis given by Dr. Novak was syncytial endometritis.

In 1950, at the Pathology Museum of the Margaret Hague Hospital, Dr. Alter of the Pathology Department showed the writer a cut-section of the brain containing a chestnut-sized

metastasis which he called syncytioma from the fact that the tumor was made up of syncytial cells.

Apparently, from the evidence gathered above, syncytioma or syncytial endometritis is not entirely benign. It can perforate the uterus and can also metastasize. However, its incidence is relatively rare when compared with either chorioadenoma destruens or choriocarcinoma.

CHORIOCARCINOMA

Choriocarcinoma or chorioepithelioma is a most malignant tumor arising from the chorionic epithelium—the syncytium and the Langhans cells. It is the result of pregnancy with which it may coexist, though most often it develops after the expulsion of the product of conception manifesting itself from a few days to as long as 3 years afterwards, Very exceptionally, choriocarcinoma begins as the direct product of conception. Four of such cases implanted in the uterus were reported by the writer.

Most of the cases resulted from uterine pregnancy. Ping-Yen Wei, et al. had 2 cases resulting from tubal pregnancy. The writer recently reported one case from an ovarian pregnancy and another case from an intraligamentary pregnancy, both of which were *ab initio* choriocarcinoma.

Incidence.—According to some authors it is very rare and occurs once in several thousand pregnancies. The incidence according to Schumann and Voegelin is 1:13,850; to King of Hongkong University Hospital, 1:3,708 pregnancies; to this writer for 1950–1955, 1:1,382 pregnancies.

As Table 2 shows, hydatidiform mole is the most frequent precursor of choriocarcinoma.

TABLE 2.—*Percentage incidence of the type of pregnancy preceding choriocarcinoma.*

(According to different authors)

Type of pregnancy	Novak and Seah, U.S. (74 cases)	Hasegawa, Japan (257 cases)	Prowiro-hardjo, et. al., Indonesia (27 cases)	Acosta-Sison Philippines (27 cases)	Ping-Yen Wei, et. al., Taiwan (17 cases)
Hydatidiform mole	39.2	67.9	70.4	63.0	35.3
Abortion	37.8	25.4	14.81	18.0	23.5
Term pregnancy	23.0	6.3	7.4	4.0	17.6
Tubal pregnancy					11.8
Ab initio				14.8	

Physical appearance.—Grossly, choriocarcinoma appears as a reddish glanular tumor rapidly growing and invading the surrounding tissues and blood vessels and with areas of hemor-

rhage and necrosis. If located in the uterus, which is the most frequent site of the primary growth, not only the endometrium is involved but also the myometrium and its blood vessels. In some cases, the endometrium is not affected and the growth resides deep in the myometrium where it may extend itself towards the broad ligaments or the peritoneal cavity. Later, it may or may not involve the endometrium.

Microscopically, it consists of masses or columns of syncytium and Langhans cells without the formation of villi surrounded by areas of hemorrhage and necrosis. Sutomo Tjokronegoro (Trawirohardjo's associate) described as diagnostic of choriocarcinoma the presence of—along with trophoblasts—what he called the "choricarcinoma triad" consisting of hemorrhage, necrosis and inflammation.

Clinical course.—If the growth is in the uterus, specially if the endometrium is involved, the most frequent symptom is bleeding. In those cases where the growth is deep in the myometrium far away from the endometrium and with the tendency to grow outwards towards the serosa, there may not be uterine bleeding. The uterus is usually larger than normal.

Metastasis is generally blood-borne and usually occurs early. In the presence of metastasis, the symptoms vary in accordance with the organ involved. The most frequent site of first metastasis is the lung which at first may not give rise to early symptoms like cough with blood-tinged sputum or the sensation of chest oppression. In some cases when the lesion is very early, it may not be revealed by an x-ray of the chest. Vaginal metastasis may be detected by palpation or by seeing a pinkish or purplish mass through the vaginal mucosa. In neglected cases, the growth may break through the mucosa and give rise to bleeding. Brain metastasis may be manifested at first by severe headache, or strabismus, sudden blindness, hemiplegia or sudden unconsciousness. This is usually followed by death.

In some cases, metastasis occurs while the product of conception is still in the uterus. This had been observed by the writer in 6 cases of hydatidiform mole and in 1 case of 5 months' normal pregnancy. Stahmann also had seen a case of advanced multiple metastatic choriocarcinoma concomitant with an almost 6 months' normal pregnancy.

As shown in Table 3 in a study of 32 cases, the writer found that the organs affected in order of frequency were

the lungs (93.75 per cent), the vagina (43.75 per cent), the liver (28.12 per cent), the kidneys (25 per cent) and the ovaries (21.87 per cent). Park and Lees also gave the same order of frequency though not the same percentage in the first 5 organs, namely, the lungs, vagina, brain, liver, and kidneys.

TABLE 3.—*Primary anatomic sites of metastasis as found by the writer in 32 cases.*

Site	Number of cases	Outcome
Lungs alone	3	Recovered
Primary lung, later other sites	11	Died
Vaginal metastasis alone	1	Recovered
Primary vaginal metastasis; later lungs	5	3 died 2 recovered
Primary vaginal metastasis; later other sites besides the lungs	4	Died
Left broad ligament alone	1	Recovered
Primary metastasis in right broad ligament then lung metastasis	1	Recovered
Primary metastasis in broad ligament; later other sites	1	Died
Primary metastasis in paremetrium; later other sites inlcuding lungs	1	Died
Primary metastasis in cervix; later other sites including lungs	1	Died
Primary metastasis in liver in conjunction with hydatidiform mole; later, other sites	1	Died
Tube and ovary-chorioepithelioma ab initio in right ovary with extensive metastasis in lungs and chorionic cell embolin many blood vessels	1	Died
Primary metastasis in right tube and ovary; later other sites	1	Died
Total	32	8 recovered 24 died

One peculiarity of some cases of fatal metastatic choriocarcinoma is that it may exist and flourish without showing the slightest trace of a primary growth in the uterus which is the seat of pregnancy that gives rise to the malignancy.

How can one explain the presence of metastatic choriocarcinoma, the sequel of uterine pregnancy without a primary tumor in the uterus? Three explanations are given to date.

One affirms that there was a primary growth in the uterus, but that after giving rise to metastasis, it regressed and became absorbed; or that it was completely removed by curettage. Novak accepted the latter explanation for his patient who died from metastatic choriocarcinoma and whose uterus showed no malignancy. He claimed to have found choriocarcinoma cells in the curetting done for early abortion in his case.

The second explanation is that malignant degeneration occurred in the trophoblastic emboli in the vessels outside the uterus.

The third explanation is based on the following facts: (*1*) Not all the chorionic cells of a given pregnancy that gives rise to choriocarcinoma—be this hydatidiform mole, abortion or term pregnancy—are malignant; (*2*) during pregnancy, it is

admitted by all that trophoblasts are often transported by means of the blood circulation to the lungs and other regions, and that these trophoblasts not being malignant eventually become absorbed and disappear; (*3*) it has been observed that metastatic choriocarcinoma may coexist with benign hydatidiform mole (as seen in 6 cases by the writer) or with a normal pregnancy (as seen by the writer in a normal 5-month pregnancy and by Stahmann in a normal almost 6 months' pregnancy).

TABLE 4.—*Incidence of metastasis in most frequently affected organs.*

Affected organs	Acosta-Sison (32 cases)	Park and Lees (295 cases)	Hunter (12 cases)
	Per cent	*Per cent*	*Per cent*
Lungs	93.75	53.55	75.0
Vagina	43.75	35.59	25.0
Brain	28.12	15.59	16.66
Liver	28.12	14.57	8.33
Kidney	25.0	11.52	8.33
Ovary	21.87	5.42	----------
Spleen	3.12	8.13	----------

Based on the above facts, the third explanation is that choriocarcinoma develops from certain specific chorionic cells that are actually or potentially malignant because of some defect (perhaps genetic or nutritional) in the fertilized ovum. If these cells happen to lodge in the uterus, as is often the case, they will give rise to a primary growth in the uterus from which metastases are disseminated. But if cells happen to be transported by the circulation either during pregnancy or after the expulsion of the product of conception, they will give rise to choriocarcinoma wherever they may lodge themselves and leaving the uterus entirely free from chorionic malignancy. This is well illustrated by Patterson's case who after recovering from metastatic intraspinal choriocarcinoma treated by surgical enucleation followed by x-ray, successfully delivered a normal term fetus three and half years afterwards.

Diagnosis.—The microscopic picture of the curettings or of the growth of the hysterectomized uterus or at autopsy will establish the diagnosis. But diagnostic curettage will give a false negative diagnosis when the malignant cells are in the myometrium beyond the reach of the curette. In such cases, the clinical signs designated as HBEs (already mentioned

under chorioadenoma destruens) or the presence of metastasis in the vagina or lungs, or an abnormal pelvic mass, in addition perhaps to a positive biologic test will point to the presence of chorionic malignancy demanding immediate hysterectomy.

As to the diagnostic value of the biologic test. In the majority of cases a fair-sized malignant chorionic growth (3 by 4 cm) or its equivalent is smaller multiple growths will give a positive frog test in the urine. However, the writer has observed in at least 2 cases of advanced choriocarinoma growths causing fatality where the frog test of the urine was consistently negative even just before death. Ping-Yen Wei also cites 2 cases of fatal choriocarcinoma where the biologic test was negative. In such cases, reliance should be given on the clinical signs and symptoms for the performance of the necessary treatment, if the patient's life is to be saved. The microscopic diagnosis may be done after the operation.

Prognosis.—The prognosis of uterine choriocarcinoma is precarious when early panhysterectomy is not performed before metastasis has occurred.

Niendorf, however, believes that choriocarcinoma cannot be so highly malignant for in the literature he found there were 81 recoveries out of 185 cases. Perhaps the patients that recovered were operated on early before metastasis had taken place or that some of them were cases of chorioadenoma destruens which were of slower growth and had lesser tendency to have widespread metastasis. We also believe in the curability of choriocarcinoma provided all the malignant cells are completely removed by early surgery before metastasis has taken place.

Novak mentioned reports of cases of spontaneous regression of choriocarcinoma growths where incomplete operation or no operation was performed. The regression of the tumor which resulted in complete recovery of the patient is explained by him as being due to an unknown factor in the general body resistance to the inroads of the disease.

Others explain such retrogression to the presence of a factor antagonistic to the trophoblasts, or to the absence of a substance calle "desmones" which is supposed to stimulate the growth of any tissue, be it benign or malignant, in any organ.

The writer observed the spontaneous disappearance of a vaginal metastasis that developed two months after the curet-

tage of hydatidiform mole. However, despite the performance of panhysterectomy and bilateral salpingo-oophorectomy, the patient died from general metastases in parts of the body other than the vagina. Pao-Chang also mentioned shadows of multiple metastases in the lungs with hemothorax and symptoms of hemoptysis which spontaneously disappeared as revealed by x-ray, the patient dying suddenly later from subdural hemorrhage. Autopsy showed regression or absence of malignant cells in the lungs.

In view of the fact that both patients died from metastases, it seems that what occurred was not dissolution of the malignant growths but the dislocation and deportation of the malignant cells from their original site to other areas.

We have not yet observed spontaneous regression of an untreated malignant chorionic tumor that ended in recovery among our 295 cases of chorionic malignancy.

Factors that affect the prognosis: (*1*) The existence of unrecognized metastasis concomitant with pregnancy; (*2*) early and widespread metastasis; (*3*) belated treatment due to failure of early diagnosis; (*4*) reliance on the verdict of a negative diagnostic curettage despite the presence of clinical signs (HBEs) specially when there is repeated or persistent bleeding, or a pelvic mass, and the positive biologic test.

With regards to the biologic test, one must be appraised of its limitations. We have seen at least 2 cases of advanced choriocarcinoma where the frog test of the urine and even of the blood were always negative even the day before death in one case.

Mortality.—Ewing is universally quoted as saying that he had never seen a patient recover from choriocarcinoma; so that the mortality is 100 per cent. Ping-Yen Wei had a mortality of 94 per cent among 17 cases. Novak and Seah had a mortality of 82.5 per cent among 74 cases. Prawirohardjo, et al., had a mortality of 66.66 per cent among 27 cases. The writer had 19 deaths or a mortality of 70 per cent among 27 cases.

A detailed study of the causes of the 19 fatalities of the writer, however, showed that in 12 cases it seemed death was due not so much to the malignancy of choriocarcinoma as to the failure to recognize the condition early enough to make the radical treatment of value in saving the life of the patient. For, in uterine choriocarcinoma, much more than in cervical

cancer, time is essential and early radical treatment must be carried out promptly if the patient's life is to be saved. There were early symptoms indicating chorionic malignancy which if treated immediately might have saved the patient's life, but neither the patient nor the physician were cognizant of their meaning. In at least two cases, the history and clinical findings including the presence of vaginal metastasis were clearly indicative as to the presence of chorionic malignancy, but treatment was withheld until after a biopsy had been made in order to be sure of the diagnosis of chorionic malignancy. And the performance of the biopsy entailed profuse bleeding requiring liberal amount of blood transfusion. In other cases, the biopsy report was not received until many days afterwards when the growth had become so far advanced that operation was no longer a safe procedure.

Studying the histories of the fatalities of others, the writer found that in many of them the interval between the cessation of pregnancy that gave rise to the chorionic malignancy and the diagnosis ranged between 3 months and 3 years and when the operation was performed not only the uterine growths were advanced but there were also present metastases elsewhere.

Six of the 19 deaths of the writer's series, however, were considered inevitable. Four were cases of ab initio chorio-carcinoma which could not be differentiated from normal pregnancy until after severe symptoms had supervened due to metastases. One was a case of generalized metastases in conjunction with an undiagnosed hydatidiform mole. The diagnosis was made only at autopsy. And one was a case of advanced liver metastasis in conjunction with benign hydatidiform mole.

Treatment.—Immediate panhysterectomy and bilateral salpingo-oophorectomy should be performed for uterine chorio-carcinoma. Huber and Hermann counsel that in young women, the tubes and ovaries should not be removed. The writer left one ovary in three young women whose ages ranged from 18 to 21 years because that ovary seemed normal. All the three cases later developed choriocarcinoma in the remaining ovary which gave rise to generalized metastases from which the women died. It is in cases of chorioadenoma destruens where normal ovaries may be safely left behind.

There is a current belief that removal of the primary growth results in the spontaneous resorption of any secondary or

metastatic growth. We have not found confirmation of such a belief in any of our cases. All metastases must be treated. If they happen to be in the broad ligament, parametrium, vagina or vulva they should be surgically removed. A solitary pulmonary nodule may be removed by partial lobectomy. If one whole lung is involved, pneumonectomy may be performed. x-ray treatment for early pulmonary metastasis has resulted in recovery. But in advanced cases, x-ray treatment alone failed to give relief.

As adjuvants to surgery, not its substitute, the following drugs had been advocated: hormones like estrogen or testosterone, and chemicals like methotrexate, nitrogen mustard, nitromin and parahydroxypropiophenone or H.365.

Others believe that the pituitary gland plays a great role in promoting the growth of choriocarcinoma by stimulating the production of the chorionic gonadotropin so that for treatment, they advocate the performance of hypophysectomy. They aver that the therapeutic value derived from estrogen or testosterone or parahydroxypropiophenone or H.365 is in their ability to suppress or to inhibit the anterior pituitary. Hypophysectomy was done in one of our cases but no beneficial or deterring effect was observed.

But is it the excess of chorionic gonadotropin that promotes the growth of the tumor and makes the patient worse? How about those cases that died from advanced choriocarcinoma who consistently showed negative frog test?

Our experience with high doses of estrogen was that this hormone failed to deter the advance of the disease.

With regards to testosterone, it has been found to be of value as an adjuvant to surgery by the writer in 3 cases of early choriocarcinoma and in 1 case of chorioadenoma destruens, by Ogawa in 4 cases of choriocarcinoma, by Genato in 2 cases of pulmonary metastases, and by Hunter in 1 case of choriocarcinoma. However, all agree that testosterone has no curative value in advanced cases. The writer lost 2 cases of advanced pulmonary metastases and Hunter lost 1 case of advanced choriocarcinoma despite large doses of testosterone.

The beneficial effect of testosterone is not so much due to its inhibitory effect on the anterior pituitary as is postulated by some as to its direct action on the malignant cells and its metabolic action favoring the assimilation of nutrients specially protein thus improving the nutrition of the patient.

The daily dose used by the writer was 100 mg and the maximum total dose that was given to a patient before the frog test of the urine became negative was 4,000 mg. The patient was well after 3 years of follow-up. No hirsutism was observed in any of the patients treated with testosterone.

Li, Herz, and Spencer found methotrexate (amethopterin) a folic acid antagonist, of value in choriocarcinoma and chorioadenoma destruens. They give intravenously an initial dose of 1 to 4.5 mg per kg body weight. Two weeks thereafter, the patient is given at 2-week intervals additional courses of 2.5 mg per kg body weight divided in 5 consecutive daily doses. Daily appraisal was made of the patient's hemogram, renal function and the presence of toxicity in the form of stomatitis, glossitis, diarrhea, proctitis, and petechial hemorrhages in the skin. These should disappear before the next course is given. Renal impairment is a contraindication to the use of the drug.

Li, et al. used 4 to 5 courses of methotrexate in 2 cases of pulmonary choriocarcinoma and in 1 case of chorioadenoma destruens, and they observed recession of the pulmonary growths, diminution of the chorionic gonadotropin and clinical improvement.

Holland also reported that with the methotrexate treatment, he observed recession of the pulmonary growths and the disappearance of a pelvic metastatic tumor in a case of choriocarcinoma.

In the Philippine General Hospital methotrexate was tried in 1 case of uterine choriocarcinoma complicated by pulmonary metastases after pelvic surgery. On the fourth day of the drug administration the patient showed stomatitis, bleeding from the lips and petechial hemorrhages in the skin so that the drug had to be discontinued. Four days thereafter the patient died. On autopsy, both lungs showed numerous multiple metastatic choriocarcinoma nodules measuring from 1.5 by 2 cm to 2 by 3 cm.

In another case of pulmonary metastasis (to be published by Manahan and Apelo) the combination of methotrexate and cobalt treatment was used. The patient, 23 months after curettage for hydatidiform mole, was operated on for partial lobectomy to remove a pulmonary nodule 2 by 2 cm which was microscopically diagnosed as choriocarcinoma. A few days after the operation, chest x-ray showed other foci of pul-

monary metastasis. Methotrexate treatment in conjunction with the cobalt exposures of the lungs was undertaken. After 2 courses of methotrexate wherein a total of 75 mg was given in the first course and a total of 135 mg in the second course with an interval of 4½ months between the courses, there was observed an apparent regression of the pulmonary shadows as revealed by x-ray. The patient also exhibited at the end of each course, specially the second one, the toxic symptoms of stomatitis, petechial hemorrhages in the skin, weakness, anorexia, and later loss of hair but these symptoms subsided after 12 days.

In the follow-up one could not use the biologic assay as a gauge of improvement in this patient for, like in the preceding case, the frog test of the urine had always been negative ever since after she was curetted for hydatidiform mole. One had to depend on her symptoms, physical findings, and x-ray examination of the chest. Eight months since the beginning of the methotrexate treatment or 3½ months after the last dose of the drug, the lung shadows showed retrogression. The patient had gained in weight, and apparently she looked well and felt well. However, after a temporary clinical remission of a few months, the pulmonary lesions revived and she finally died from pulmonary and brain metastases 8 months after the last dose of methotrexate or 3 years after she was curetted for hydatidiform mole.

Since January, 1959, however, methotrexate treatment resulted in apparently complete recovery of 2 cases of pulmonary metastatic choriocarcinoma and 3 cases of chorioadenoma destruens. So that the drug, when properly administered offers much hope in the cure of chorionic malignancy.

Anderson, et al. reported a case who 25 days after undergoing left pneumonectomy for advanced metastatic pulmonary choriocarcinoma of the left lung showed metastases in the right lung. The treatment given was the daily intravenous injection of 5.7 mg of nitrogen mustard for 5 consecutive days. Then, after an interval of 27 days' rest, the daily injection was resumed for 4 days increasing the dose to 6 mg. The metastases disappeared and the patient got well and continued to be so at the end of one-year follow-up.

Beecham, et al. used for treatment of the metastatic pulmonary choriocarcinoma the combination of nitrogen mustard and x-ray exposure. In 1 case, there was retrogression of the

growth. In another case, however, the treatment failed to save the life of the patient.

The rationale of the use of the nitrogen mustard is that it is supposed to have a nucleotoxic effect on tumor tissue and that it potentiates the radiation effect of the x-ray in the treatment of pulmonary metastasis.

Wei found good result from the intravenous injection of a combination of nitrogen mustard (40 mg) and nitromin (300 mg) for pulmonary metastasis from a primary tumor of chorioadenoma destruens. He also used the above combined drugs in 6 cases of pulmonary metastasis from choriocarcinoma. In 2 cases, he obtained minimization of the pulmonary shadows but both eventually died from extensive metastases.

Ishizuka of Osaka University reported (at the International Symposium on Cancer Chemotherapy in Tokyo on October 24 to 28, 1957) nineteen cases of chorionic malignancy treated with nitromin. Eleven cases were cured and 8 died. Two of the fatal cases temporarily responded by negativity of the pregnancy test, and disappearance of metastatic lesions but later died from recurrences. Ten of the cured patients were cases of chorioadenoma destruens. Of the 7 cases of choriocarcinoma, only 1 was cured.

Perrault of France reported success in the treatment of choriocarcinoma with the use of parahydroxypropiophenone or H.365.

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ILLUSTRATIONS

Plate 1

Fig. 1. Malignant mole or chorioadenoma destruens in conjunction with choriocarcinoma. Patient died from metastatic pulmonary choriocarcinoma.

2. Chorioepithelioma uterus, anterior view, showing spots where the tumor is about to go through the peritoneal layer. The patient had also metastasis in the lungs. Patient died.

3. Perforative chorioepithelioma of the uterus showing bilateral cystic ovaries. Patient had metastasis over the rectum and in both lungs. The sites of metastasis were treated by x-ray. Patient recovered.

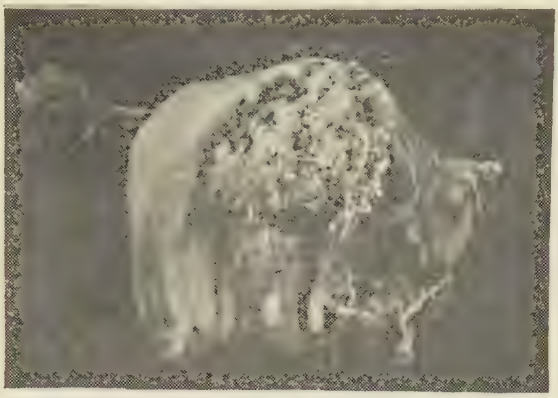

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PLATE 1.

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SECOND TEN-YEAR INDEX. THE PHILIPPINE JOURNAL OF SCIENCE, vol. 11 (1916) to vol. 28 (1925). Compiled by Winifred I. Kelley. Bureau of Science Monograph 26. Paper, 382 pages. Price, $2.00 United States currency, postage extra.

CHECKLIST OF THE ANTS (HYMENOPTERA: FORMICIDÆ) OF ASIA. By J. W. Chapman and S. R. Capco. Institute of Science and Technology Monograph 1 (1951) new series. Paper, 327 pages. Price, $2.00 United States currency, postage extra.

NOTES ON PHILIPPINES MOSQUITOES, XVI. GENUS TRIPTEROIDES. By F. E. Baisas and Adela Ubaldo-Pagayon. Institute of Science and Technology Monograph 2 (1952) new series. Paper, 198 pages with 23 plates and 4 text figures. Price, $2.50 United States currency, postage extra.

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